INTRASPECIFIC VARIATION IN RESOURCE USE, DORMANCY INVESTMENT, AND COMPETITIVE ABILITY IN THE FACULTATIVE PARTHENOGEN DAPHNIA PULICARIA

BY

JOHN WILLIAMS CRAWFORD

DISSERTATION

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Doctoral Committee:

Professor Carla E. Cáceres, Chair and Director of Research
Professor Andrew V. Suarez
Associate Professor Rebecca C. Fuller
Assistant Professor Katy D. Heath

ABSTRACT

Ecological studies highlight that many species and most populations maintain high degrees of intraspecific (within species or within population) variation in a wide variety of life-history traits. This pattern prompts the question: how important is this variation? This is a question that is especially motivating for evolutionary ecologists as we are interested in how intraspecific variation influences evolutionary dynamics. While the maintenance of intraspecific variation is especially common in species that reproduce sexually, it is also common in clonal (and partially clonal) species. In my dissertation, I use the facultatively parthenogenetic zooplankton *Daphnia pulicaria* to address several questions related to the breadth and consequences of intraspecific variation.

Freshwater zooplankton species are important subjects for ecologists. In particular, crustaceans in the genus *Daphnia* have been studied due to their importance as keystone species in many freshwater lakes. They provide ecosystem services including moving resources from the primary producers up to higher trophic levels and have served as models for grazers in other systems. Decades of research on *Daphnia* demonstrate substantial interspecific (between species) and intraspecific (within species) variation in how individuals respond to available food resources. These studies targeted both spatial variation in the quality and quantity of algal resources between neighboring lakes and temporal variation (including changes due to decades of eutrophication). This prior research has shown that there is interspecific variation in the ability of species to capitalize on different resource quantities and qualities. One limitation of prior research in this system is that it has concentrated almost exclusively on interspecific variation in resource acquisition and allocation and has largely ignored intraspecific variation within and among populations.

Variation in resource quality in natural systems along with trade-offs between and within populations in response to resources makes the *Daphnia*-algae system ideal for examining intraspecific variation in key life-history traits. Prior work on *Daphnia* suggests both an interspecific and intraspecific trade-off in response to resource quality between “powerful” and “efficient” species and individuals. In several studies, Alan Tessier and colleagues found that some *Daphnia* species were better able to capitalize on rich resources and that these “powerful” individuals maximized their growth on both high-quantity and rich-quality resources but were

"sensitive" to the decline in resource quality or quantity. Other "efficient" species were better able to maintain their growth on low quantity or poor-quality resources, and were not sensitive to the decline in resource quantity or quality. Further work by Spencer Hall and colleagues extended this examination to intraspecific variation in individual ability to use resources of different qualities. They found similar variation in terms of powerful and efficient strategies occurring among individuals of *Daphnia ambigua*. My research goal was to examine how intraspecific variation in response to resource quality may (1) be influenced by the population or season from which an individual was collected, (2) influence the likelihood of an individual to invest in sexually-produced dormant offspring, and (3) influence competitive ability.

In the first chapter, I explore intraspecific variation in response to resource quality in 143 clones from six populations of *Daphnia pulicaria* in Michigan. In freshwater lakes, the quality of algal resources varies seasonally as the rich-quality resources of the spring are replaced by poor-quality resources in the summer. Concurrently, there is a decline in population density of large bodied grazers (such as *D. pulicaria*) through the combined effects of competition for resources, predation, and parasitism. In some "non-persisting" populations the decline in density is so dramatic that populations are reduced to undetectably low levels by the summer. As individuals from these non-persisting populations do not experience poor-quality resources in the summer, I predicted that these individuals would grow relatively poorly on poor-quality algal diets in the laboratory (i.e. would be sensitive to changes in resource quality). I also predicted populations that persisted through the summer would have fewer individuals who were sensitive to the decline in resource quality as these individuals are exposed to both rich- and poor-quality resources in the field. Although I found significant variation in response to resource quality, my results did not support the prediction that sensitivity to resource quality is greater in the non-persisting populations. I further examined the genetic consequences of resource sensitivity by looking at turnover in clone identity. I predicted a turnover in genotypes between spring and summer in the persisting populations as more efficient genotypes persevered and the sensitive genotypes were selected against. Although there was evidence for rapid evolution between spring and summer, my results did not support the prediction that the distributions of growth rates were driven by changing qualities of resources in the field.

Daphnia exhibit considerable intraspecific variation in the likelihood of investing in sexually-produced dormant offspring, but why some genotypes are more likely to invest in

sex/dormancy is less understood. For many *Daphnia* species (including *D. pulicaria*), sexual reproduction is the only means of producing dormant offspring; therefore, investment in sex/dormancy can be viewed as a cost-benefit analysis in which individuals forgo current production of numerous clonal daughters for the future hatching of fewer but sexually-produced daughters. In chapter two, I examined whether this variation is due to the ability of a genotype to grow on different qualities of resources, an indication of current fitness. Using 121 of the clones from chapter one, I assessed the likelihood of a genotype to invest in sexual reproduction and dormancy by quantifying investment in clonal daughters, clonal sons, and haploid eggs awaiting fertilization. Although the observed variation suggests other factors contribute to the likelihood of allocating to sex/dormancy, I found that individuals with lower mean growth had higher investment in sexually-produced dormant offspring. There are three possible explanations for this finding. First, dormancy constitutes a temporal escape from poor environmental conditions such as the onset or expected onset of higher competition, predation, and parasitism. Second, individuals with low current fitness may be more likely to invest in sexual reproduction for the potential fitness benefits to their offspring. Or third, the investment in sexually-produced dormant offspring could be for the joint benefits of both sexual reproduction and dormancy.

In chapter three, I explored whether the laboratory-assessed sensitivity to resource quality (from chapter one) drives competitive dynamics and predicts growth on field-collected resources. I used three genotypes that were sensitive to changes in resource quality and three that were efficient and maintained equivalent growth on both rich- and poor-quality resources. As resource quality in the field progresses through a seasonal succession from rich-quality algae in the spring to poor-quality, toxic, and/or digestion resistant algae in the summer, my first prediction was that sensitive individuals would be able to grow better (have higher expected fitness) on spring- and poorly on summer-collected resources. My results did not support the prediction that laboratory-assayed sensitivity predicted an individual's growth on field-collected resources. Secondly, I predicted that the outcome of a laboratory competition assay would mirror these results; high sensitivity genotypes were predicted to perform better in rich-quality competition diets. My results show that individuals of both sensitivities reached equivalent densities in both diet treatments and there was no competitive advantage in the 21-day experiment. Despite the difference in performance by these genotypes on rich-quality resources documented in chapter one, sensitivity to resource quality does not appear to drive competition dynamics. Instead, high

overall densities and resource limitation may have constrained population growth and the effects of intra-strain and inter-strain competition may have had an equivalent effect in this low volume, short-term, and high-density experiment. Although sensitivity to resource quality is not governing performance on field-collected resources or in short-term competition, I suggest that other factors such as a genotype's ability to maintain growth on poor-quality resources may be a more important metric for future studies seeking to predict individuals' growth in the field or longer-term competitive ability.

In conclusion, I demonstrated several interesting results in the study of intraspecific variation. First, there is significant variation in response to resource quality both between and within populations of what has been previously described as a generalist grazer. Second, this intraspecific variation in growth contributes to an individual's likelihood of investing in sexually-produced dormant offspring. Third, while my analysis failed to link sensitivity to resource quality with competitive ability, there is intraspecific variation in other growth traits that should be explored. My results indicate two important factors that evolutionary ecologists should continue to consider. First, using the mean trait of a species misses a lot of interesting and potentially important variation. Secondly, variation in suites of ecologically important traits can influence other suites of traits. To better understand biological phenomena, we must consider the importance of intraspecific variation and the joint-effects of variation on other suites of traits.

To Dr. John Williams Schaller (1927 – 2014),

A great man, doctor, philanthropist, and scientist with unending curiosity.

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Chapter 1: Intraspecific variation in response to changing resource quality in six populations of *Daphnia pulicaria.*

Abstract

Temporal variation in resource quality has the potential to maintain diversity in consumer traits within and among populations of generalist grazers. To quantify the distribution of this variation, we sampled six populations of the freshwater cladoceran *Daphnia pulicaria* and asked whether population persistence from spring to summer is predictive of growth rate on diets of varying quality. In populations that persist year-round, genotypes have the potential to experience diets that vary in quality whereas in those populations that are only abundant in the spring (non-persisting populations), genotypes primarily encounter rich-quality resources. Laboratory assays allowed us to test two predictions: (1) that genotypes from non-persisting populations would have lower growth rates on poor-quality foods compared to genotypes from populations that persist throughout the year and (2) in the persisting populations, clonal selection from spring to summer would shift the distribution of genotypes from those that are able to grow rapidly on the rich-quality spring resources to those that are better at growing on the poor-quality summer resources. Although the six populations are differentiated with respect to average growth rate, we did not find a significant effect of permanence type on growth rate, nor did we find a diet-by-type interaction. In the three persisting populations, we found evidence for clonal turnover from spring to summer, but we did not find strong evidence supporting the hypothesis that changing resources drives the distribution of growth rates. Instead, the variation we found in growth rate among clones is likely maintained by the complex food web dynamics clones experience in these lakes.

Introduction

Despite the potential for strong selection, consumer populations often maintain persistent genetic variation in traits associated with resource acquisition and allocation (Kirk 1997, Boon et al. 2007, Allen et al. 2012). For example, individuals that grow exceptionally well on rich-quality resources but not as well on poorer-quality resources (i.e., 'powerful' genotypes) may co-

occur with more 'efficient' consumers that grow relatively better on poorer resources but cannot exploit richer resources to the same extent as powerful genotypes (Odum and Pinkerton 1955, Kobe et al. 1995, Raubenheimer and Simpson 1996, Tessier et al. 2000, Hall et al. 2012). Sufficient diversity in resource acquisition and allocation strategies of consumers may be maintained by environmental variation in resource quality via this "power-efficiency" tradeoff (Reznick et al. 2000, Tessier et al. 2000, Kassen 2002). Both powerful and efficient strategies may be simultaneously maintained within populations: powerful individuals can capitalize on rich-quality resources but temporal and spatial heterogeneity of resource quality means that powerful individuals may be selected against when resource quality declines. As a result of this tradeoff, consumer species that are often thought of as generalist grazers may in fact be composed of individual specialists (Tinker et al. 2012, Terraube et al. 2014). A central question then becomes, what factors determine the distribution of these specialized consumer phenotypes within and among populations?

Freshwater lakes offer an excellent system in which to explore this question. Many lakes exhibit predictable changes in the quality of algal resources from rich-quality in the spring to poorer-quality in the summer (Sommer et al. 1986, Sommer et al. 2012). Temporal changes in resources are often linked with the population dynamics of the main consumers (such as the large-bodied grazers of the genus *Daphnia*) with grazer population density increasing with rich-quality resources in the spring and declining with the reduced resource quality of the summer (Goulden and Hornig 1980, Tessier 1986, McCauley and Murdoch 1987). Field patterns demonstrate that the extent to which *Daphnia* populations decline from spring into summer differs between lakes; some lakes can only support populations of large-bodied grazers for part of the year, whereas in others large grazers remain active year-round (Cáceres and Tessier 2004a, Allen and Lynch 2008, Hamrova et al. 2011). Demographic differences between these "persisting" and "non-persisting" populations in turn can translate into among-lake variation in the strength of clonal selection between seasons (Chesson and Huntly 1997, Kingsolver et al. 2001). Among-lake differences in clonal selection have implications for how consumer traits, such as growth rate, are distributed in space and time.

We combined laboratory growth assays with quantification of clonal richness to test hypotheses regarding the role of population permanence (persisting vs. non-persisting) and collection season (spring vs. summer) on the distribution of growth rates in *Daphnia pulicaria.*

Specifically, we included three persisting populations with active *D. pulicaria* in the water column throughout the year and three non-persisting populations that only have detectable *D. pulicaria* populations in the spring and find refuge in the egg bank during the summer months (Table 1.1; see also Cáceres and Tessier 2004a). Previous research in these and other lakes have documented considerable variation in the ability of individual genotypes to exploit richer vs. poorer quality resources (Weider et al. 2005, Brezeziński and Von Elert 2007, Brezeziński et al. 2010, Hall et al. 2012). Some genotypes, the so-called "powerful" or "sensitive" genotypes, grow quickly on rich-quality resources but are extremely sensitive to declining resource quality and suffer severe fitness reductions when grown on poor-quality resources. Conversely, other genotypes have nearly equivalent growth rates on both richer- and poorer-quality resources. These "efficient" genotypes can never out-grow sensitive genotypes on rich-quality resources, but suffer little to no fitness reductions on poorer-quality resources. Populations may contain both powerful and efficient individuals but the proportion of these strategies may depend on the resource regime of the lake. These two growth strategies can manifest as a trade-off with some individuals better able to capitalize on good conditions and others better able to maintain growth in poor conditions (Tessier and Consolatti 1991, Via 1991, Pigliucci and Schlichting 1998, Tessier et al. 2000, Hairston et al. 2001, Tessier and Woodruff 2002, Brzezinski and Von Elert 2007).

Given that rich-quality resources are common only in the spring and these resources favor the fast-growing but sensitive genotypes, we tested two main predictions. First, we predicted that for genotypes collected in the spring when resource quality is richest, non-persisting populations would have a higher prevalence of sensitive genotypes compared to populations that persisted throughout the year (as measured via a diet-by-type interaction). This prediction is based on the hypothesized trade-off of being able to exploit rich vs. poor resource quality; in non-persisting populations, genotypes rarely experience the poor-quality resources of the summer and therefore the slower growing efficient genotypes should be rare as powerful genotypes have an advantage when feeding on rich-quality resources. Our second prediction focused only on the three lakes in which the population persists at detectable densities year round. We predicted that the distribution of genotypes would shift (presumably via clonal selection) from being dominated by highly sensitive genotypes in the spring to slower growing, less sensitive, genotypes during the summer. These more efficient generalist genotypes have

lower growth rates on rich-quality spring resources (and are therefore predicted to be less abundant in spring) but suffer less of a fitness reduction and may be superior competitors on the poorer-quality summer resources (Lynch 1984, Tessier and Woodruff 2002, Delmotte et al. 2002).

Methods

Field Collection and Genotyping

To test our predictions, we sampled six lakes in central Michigan near the Kellogg Biological Station (Barry and Kalamazoo Co.; Table 1.1). Three lakes maintained active populations of *Daphnia pulicaria* throughout the year ("persisting populations" – Bassett, Bristol, and Warner; Cáceres and Tessier 2004a), whereas by midsummer *D. pulicaria* largely disappears from the water-column in the other three lakes ("non-persisting populations" – Baker, Cloverdale, and Little Long; Cáceres and Tessier 2004a). We sampled all six populations in spring (May) 2012 and 2013. We also sampled the three persisting populations in summer (August) 2011 and 2012. On each sampling date, we collected live *D. pulicaria* with triplicate bottom-to-surface tows from the deepest region of the lake and isolated >100 live female *D. pulicaria* per lake into individual cultures. Additional *D. pulicaria* individuals were preserved in ~95% EtOH for subsequent molecular analysis.

We used six microsatellite loci to assess genetic variation in the established iso-female lines (Dp 27, 78, 102, 196, 433, 461; Colbourne et al. 2004, Cristescu et al. 2006, Allen et al. 2010, Holmes et al. 2016). To capture the maximum genetic and phenotypic variation present in these populations, our experiments were conducted on a sub-set of clones from each population that were unique at these six microsatellite loci compared to other clones collected at the same time from that population. To assess which multilocus genotypes (MLGs) were present in both May 2012 and August 2012, we used the Poppr R package for population genetics (R package Poppr, R version 3.1.3, R Core Team 2013). Full methods for genotyping and sample sizes can be found in Appendix A.

Laboratory Assays

To assess the distribution of growth rates in space and time, we conducted juvenile growth rate (JGR) assays (Tessier and Goulden 1987, Lampert and Trubetskova 1996, Tessier and Woodruff 2002, Allen et al. 2010). In *Daphnia*, JGR is a common fitness proxy and a correlate of per capita growth rate; JGR also allows us to test response to resource quality by determining how much an individual grows on rich- and poor-quality resources (Tessier and Goulden 1987, Lampert and Trubetskova 1996). To standardize maternal effects, we first conditioned our clonal lines for three generations according to Lynch and Walsh (1998). *Daphnia* were maintained individually in 150ml of filtered lake water and were fed high concentrations (>2mg/L dry weight) of *Ankistrodesmus falcatus* every two days. From these cultures, third generation offspring became mothers of the neonates used in the JGR assay. In total, we assayed 146 genotypes (minimum 11 per season per population; Table B.1) on equal concentrations (2 mg/L dry weight, fed daily) of two diets, *Ankistrodesmus falcatus* (rich-quality; simulating spring resources) or *Oocystis* B (DeMott et al. 2010). *Oocystis* B provides a poor-quality summer resource proxy because, like many dominant summer green algae in our study lakes, it has an inducible, digestion resistant sheath (DeMott et al. 2010, Hall et al. 2012).

To begin an assay, adult females from each genotype with late-stage eggs were isolated in filtered lake water. After at least six, but no more than 20 hours after birth, half of the neonates from each genotype (haphazardly selected, $N > 5$) were dried in a 60°C oven overnight for analysis of initial weights. Remaining neonates were haphazardly dispensed into beakers at a non-crowded density (not exceeding three individuals per 200mL). Each replicate of each genotype-by-diet combination included a minimum of three beakers per resource treatment, each containing three individuals per beaker. Experimental animals were grown in a 20°C environmental chamber for five days on a 14[light]:10[dark] photoperiod. After five days of growth, experimental animals were bathed in filtered lake water and dried at 60°C overnight for final dry mass. Dry mass was assessed using a Mettler Toledo UMX2 microbalance (c. tenth of a microgram). During experimental setup, take down, and weighing, we lost some individuals due to death or carapace damage. Prior to analysis, any replicate with fewer than five individuals per resource treatment were removed. Final sample sizes for each population and season of collection can be found in Table B.1. Juvenile growth rate was calculated as in Tessier and Goulden (1987):

$$\text{JGR} = \frac{\ln(\text{mass on day five}) - \ln(\text{mass on day zero})}{\text{days of growth}}$$

Statistical Analysis

To test whether non-persisting populations had greater mean sensitivity to resource quality (indicating that they were more powerful), we analyzed the juvenile growth rate of the spring-collected genotypes from all six populations. We used a nested ANOVA with persistence type (persisting or non-persisting), population of collection, and diet quality (i.e., rich-quality *Ankistrodesmus* vs. poor-quality *Oocystis* B) and their interaction as fixed factors (PROC GLM, SAS version 9.4). The interaction with diet quality determines whether populations of population types differed in their sensitivity to resource quality. Sensitivity refers to the difference in juvenile growth rate between the two diets; as powerful genotypes are also sensitive to changes in resource quality, we can use this metric to determine whether non-persisting populations have more powerful/sensitive individuals. To determine if there was a difference between seasons within the persisting populations, we examined the juvenile growth rate of the three persisting populations via ANOVA by including diet quality, population of collection, season of collection (spring- vs. summer-collected genotypes), and all interactions as fixed effects. A significant season-by-diet interaction indicates that individuals collected in different seasons differed in their mean sensitivity to resource quality; therefore, we use this interaction to determine if more powerful individuals are common in the spring compared to the summer.

Results

In the field populations, we detected 153 unique multilocus genotypes with our six microsatellite markers. Clonal richness (proportion of unique clones) in each lake ranged from 0.3-0.95 for the spring collection (Table B.1), but year-to-year variation in several populations resulted in no difference in clonal richness between the persisting and non-persisting populations (Welch Two-Sample T-Test, $t=0.57$, $p > 0.05$). The summer-collected clones from the three persisting populations also showed considerable among-year variation in clonal-richness for two of the three populations, resulting in no among-population ($F_{2,6} = 2.41$, $p > 0.05$), among season

($F_{1,6} = 2.37$, $p > 0.05$), and no season-by-population interaction ($F_{2,6} = 1.13$, $p > 0.05$) in clonal-richness.

Despite the absence of significant differences in clonal richness among populations or seasons, we found significant phenotypic diversity in growth rates in the six populations (Fig. C.1). Average growth rates differed significantly among populations, but contrary to our prediction, the sensitivity of spring-collected genotypes did not differ between persisting and non-persisting populations (Table 1.2). We found no difference in mean growth rate or sensitivity to resource quality (measured as the diet-by-type interaction) among persistence types (Fig. 1.1A). Not surprisingly, average growth rate was higher on the rich-quality diet than on the poor-quality diet, although there was overlap in growth rates achieved on the two diets (Fig. C.1B and Fig. C.1C). Additionally, rather than finding a tradeoff in the ability to exploit the two diets, growth rates of the individual genotypes on the two diets were positively correlated (Fig. 1.2).

Only the three persisting populations allowed for a comparison of growth rate and sensitivity between spring- and summer-collected clones. There was evidence for significant variation in growth rates (Fig. 1.1B, Table 1.3), but genetic variation within lakes obscured any seasonal differences. Nevertheless we did find evidence for turnover in genetic composition between spring and summer collections (Fig. 1.3). In Bassett Lake, there was no overlap in genotypes between the 111 spring-collected individuals and the 53 summer-collected individuals (Fig. 1.3A). Bristol and Warner Lakes had less seasonal clonal turnover and each population shared genotypes collected in both spring and summer (Fig. 1.3B and Fig. 1.3C). In Bristol Lake, all clones were <10% of the population in both seasons, whereas the other two populations had at least one dominant clone that made up >20% of the population.

Discussion

We hypothesized that selection on traits related to a genotype's ability to exploit resources of varying qualities would lead to predictable patterns of *Daphnia* growth rates within and among populations. Specifically, we predicted that fast growing genotypes that are sensitive to declines in resource quality would be more common during the spring in non-persisting populations relative to persisting populations. Additionally, within persisting populations, we

predicted that spring-collected clones would have higher growth rates and suffer greater fitness reductions when fed poor-quality diets than summer-collected clones. For clones collected in the spring, persistence type (persisting vs. non-persisting) did not explain the variation in juvenile growth rate nor sensitivity to diet quality (diet-by-type interaction), despite the maintenance of substantial variation in growth rates and the differentiation of populations. In the persisting populations, we found no evidence for the relationship between clonal growth rate and season. Our results suggest that seasonally fluctuating resources alone cannot explain the maintenance of variation in clonal growth rates.

Multiple species of *Daphnia* exhibit intraspecific variation in growth rates in response to changing resource quality and there is evidence for tradeoffs in their ability to exploit richer- vs. poorer-quality resources (Hutchinson and Bowen 1947, Wiens 1976, Sommer et al. 1986, De Stasio et al. 1995, Hairston et al. 2001, De Mott and Tessier 2002, Weider et al. 2005, Brzeziński and Von Elert 2007, Allen et al. 2010). We documented a considerable range of growth rates across populations and seasons of collection (0.18-0.53 μg/μg/day on *Ankistrodesmus* and 0.16-0.37 μg/μg/day on digestion resistant *Oocystis*). Nevertheless, our predictions to explain this variation were not well supported. Our hypotheses were based on an assumption that clonal selection (both within and among populations) would be driven by changes in resource quality. There are at least three additional relevant variables that change seasonally in lakes that our laboratory experiments did not explore: predation, parasitism, and temperature. These biotic and abiotic factors have been shown to influence clonal growth rate in *Daphnia* (Spitze 1991, Giebelhausen and Lampert 2001, Hall et al. 2007, Hart and Bycheck 2011, Walsh and Post 2011, Walsh and Post 2012, Burns 2013, Civitello et al. 2015). Hence, we can think of at least two explanations for the lack of support for our hypotheses: (1) trade-offs with other fitness-related traits (such as coping with predation, parasitism, or temperature) over-ride a power-efficiency trade-off in these populations and/or (2) our representative "poor-quality" diet was not of poor enough quality to reveal this trade-off in the lab, at the intermediate temperature we employed.

More than a half-century of research on lakes and ponds worldwide has clearly documented the influence that predation can have on *Daphnia* growth rates (reviewed in Hart and Bycheck 2011). For example, fast growing (and usually larger-bodied) genotypes often have higher death rates due to predation by planktivorous fish (Brooks and Dodson 1965, Walsh and Post 2011, Walsh and Post 2012). The slow growth rate of genotypes from one of the non-

persisting populations (Little Long) on both diets may be correlated with the very high predation intensity found in this oligotrophic lake that does not thermally stratify, meaning that this population does not have a refuge from fish predation (Gerrish and Cáceres 2003, Cáceres and Tessier 2004a). Summer refuge is also eliminated in the other two non-persisting populations (Baker and Cloverdale) as the hypolimnion becomes anoxic by midsummer (Cáceres and Tessier 2004a). In the three persisting populations, the availability of a deep-water refuge allows populations to persist year round (Cáceres and Tessier 2004a). In stratified lakes such as these, clones with different traits are known to co-exist via spatial habitat partitioning across the thermocline (Weider 1985, Tessier and Leibold 1997, Boeing et al. 2004, Seda et al. 2007a, Seda et al. 2007b, Dawidowicz et al. 2013, Meyer 2016). Clones that remain in deep water experience reduced mortality by planktivorous fishes but can have much longer development times. Trade-offs between foraging efficiency and predation risk are well known (Lima et al. 1985, Brown et al. 1994, Pekarsky et al. 2008, Kotler et al. 2010) and a better understanding of how the growth rates we measured are correlated with predation risk in the field could help to explain the maintenance of genetic variation in growth rate.

The possibility of clones co-existing via habitat partitioning raises a second potentially relevant factor that we did not explore: temperature. Among-clone differences in thermal responses could contribute to the maintenance of variation both among populations and among seasons. In thermally stratified systems, deep water clones experience much lower average temperatures than do metalimnetic or migrating clones; and in the permanent systems, spring clones were collected from much cooler temperatures than migrating or epilimnetic summer clones. We have no information about where our summer clones were residing in the water column, but our assays likely include genotypes with different migration patterns (e.g., De Meester 1994, Tessier and Leibold 1997). Several previous studies in *Daphnia* have found significant diet-by-temperature and genotype-by-temperature interactions in multiple traits, including juvenile growth rate (Mitchell and Lampert 2000, Chopelet et al. 2008, Przytulska et al. 2015). For example, Giebelhausen and Lampert (2001) used a four temperature by four resource concentration factorial experiment to measure the juvenile growth rate and other life history traits of spring- and summer-collected clones of *D. magna* and found a much stronger temperature response when resources were not limiting. All of our assays were conducted at 20°C, it is therefore possible that we would have found more support for our hypotheses had we

also included a spring-like temperature, but the large number of clones we included in this assay restricted us to a single temperature.

Parasites are a third major factor that we did not consider here that could influence the distribution of growth rates in space and time. In *Daphnia pulicaria*, common parasites include the fungal *Polycaryum laeve*, the bacterium *Spirobacillus cienkowskii,* an as-yet unidentified bacterial infection, and the microsporidians *Gurleya* sp. and *Larssonia obtusa* (Johnson et al. 2006a, Duffy et al. 2010, Cáceres et al. *Unpublished Data*). These obligately-killing parasites are encountered while foraging; therefore, the rate at which an individual feeds is correlated with their exposure rate to these deadly pathogens (Hall et al. 2007, Hall et al. 2010, Hall et al. 2012, Garbutt and Little 2017). There are numerous direct and indirect effects of diet, temperature, and predators on mortality rates due to parasites (Johnson et al. 2006b, Cáceres et al. 2009, Duffy et al. 2011, Bertram et al. 2013) and parasite-induced mortality can be a major selective force in daphniid populations (Duffy et al. 2008, Wolinska and Spaak 2009, Duffy et al. 2012). The interactions among competition for resources of varying quality, predation, parasitism, and perhaps even three-way trade-offs (e.g., Edwards et al. 2011) are likely maintaining the diversity in growth rates that we observed.

In addition to the existence of trade-offs influencing our results, a second possibility is that our choice of diet treatments did not fully capture the annual decline in resource quality. We used digestion resistance as our proxy for poor-quality algal resources experienced by *Daphnia* in the summer (as in DeMott et al. 2010, Hall et al. 2012). However, there are other metrics for algal food quality such as toxicity, stoichiometry (including C:P), limiting nutrients, and essential fatty acids; all have been shown to influence the growth rates of *Daphnia* (DeMott and Tessier 2002, Hairston et al. 1999, Hairston et al. 2001, Sterner and Elser 2002, Jeyasingh et al. 2003, Archarya et al. 2006, Fey and Cottingham 2011). It is less common for studies to simultaneously address multiple metrics of resource quality (DeMott and Tessier 2002, Becker and Boersma 2003, Ravet and Brett 2006), but had we chosen a different diet quality metric or used natural seston, we may have observed different patterns.

We found some evidence for a change in the genetic structure of populations from spring to summer. This change was especially obvious in Bassett where none of the genotypes collected in the spring of 2012 were also collected in the summer of 2012. Despite this complete turnover in unique multilocus genotypes between spring and summer, there was no difference in

sensitivity to resource quality and spring- and summer-collected individuals performed equivalently on the two diets used in this assay. As a result, we found no strong evidence for clonal selection based on response to diet quality. Brzeziński et al. (2010) made a similar prediction regarding clonal turnover driven by changes in resources. They found that spring- and summer-collected clones of the *Daphnia longispina* group (*D. hyalina* and *D. galeata-hyalina* hybrids) differed in performance but, as is the case with our study, found no diet-by-season interaction, which means that clones always grew better on richer-quality resources. Brzeziński et al. (2010) also noted that predation by planktivorous fish may contribute to the variation in daphniid growth rates.

In quantifying genetic and phenotypic variation in six populations, we found considerable within-species variation, as well as evidence for population differentiation in growth rates. Although we report some evidence of temporal changes in the dominant genotype in persisting populations, our results indicate that a simple trade-off in response to resource quality is insufficient to explain the variation in growth rates. Nevertheless, this among-individual variation can influence both ecological and evolutionary dynamics, thus an understanding of its distribution has the potential to improve our ability to explain the complexity of natural systems (Bolnick et al. 2003, Bolnick et al. 2010, Fridley & Grime 2010, Violle et al. 2011, Forsman & Wennersten 2015).

Tables and Figures

Table 1.1. Among lake differences in morphometry and productivity influence the annual population dynamics of *Daphnia pulicaria.* Values for total phosphorus (TP, µg $P\text{-}PO_4$ / L) are from spring turnover averaged for 2–5 years (Tessier & Woodruff 2002; Cáceres & Tessier 2004b). Coefficient of variation (CV) of *D. pulicaria* density fluctuations is calculated from weekly density estimates (Cáceres & Tessier 2004a).

Lake	Surface Area (ha)	Max Depth (m)	Spring TP (µg L^{-1})	CV of density change
Non-persisting populations				
Baker	23.8	9	25	38
Cloverdale	44.1	15	15	36
Little Long	67.7	9.5	8	35
Persisting populations				
Bassett	18.8	11.5	15	17
Bristol	62.8	15	11	18
Warner	28.4	14	10	21

Table 1.2. Nested analysis of variance (ANOVA) of the juvenile growth rate (JGR) on rich (*Ankistrodesmus*) and poor (*Oocystis*) quality diets for spring-collected genotypes from both types (persisting and non-persisting) of populations. Diet quality, persistence type, and population (nested in persistence type) are treated as fixed effects. Degrees of freedom (d.f.), F-ratios, and P-values are provided.

Effect	d.f.	*F*	*P*
Diet	*1, 196*	*434.72*	*<0.0001*
Type	1, 4	3.98	0.1168
Population (Type)	*4, 196*	*5.16*	*0.0006*
Diet * Type	1, 4	0.34	0.59
Diet * Population (Type)	4, 196	0.38	0.82

Table 1.3. Analysis of variance (ANOVA) fit to juvenile growth rate (JGR) for spring- and summer-collected genotypes from persisting populations fed rich (*Ankistrodesmus*) or poor (*Oocystis* B) quality diets. Diet quality, population, and season of collection are treated as fixed effects. Degrees of freedom (d.f.), F-ratios, and P-values are provided.

Effect	d.f.	*F*	*P*
Diet	*1, 174*	*377.52*	*<0.0001*
Population	*2, 174*	*6.18*	*0.0026*
Season	1, 174	0.38	0.537
Diet * Season	1, 174	0.66	0.416
Diet * Population	2, 174	0.32	0.726
Season * Population	2, 174	1.80	0.168

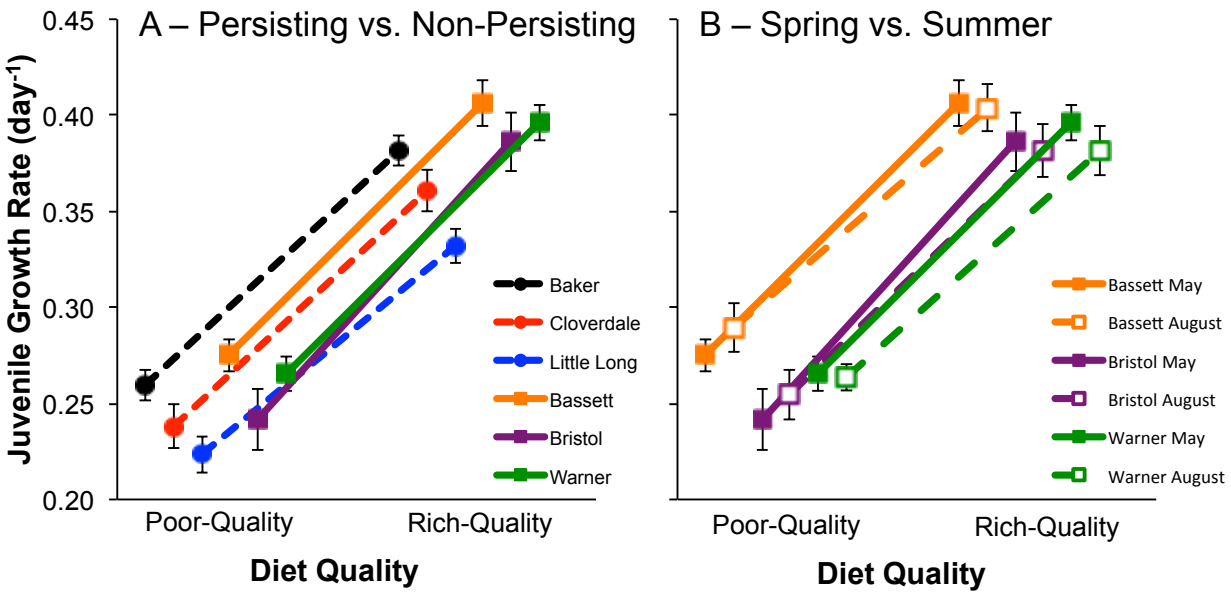

Figure 1.1. Performance of clonal genotypes on rich (*Ankistrodesmus*) and poor (*Oocystis*) quality diets, indexed as juvenile growth rate. (A) Juvenile growth rates (JGR) for the spring-collected clones from the six populations. Key: non-persisting populations: circles, dashed lines; persisting populations: squares, solid lines. (B) Juvenile growth rates (JGR) for the spring- and summer-collected clones in the three persisting populations. Key: Spring-collected: solid squares, lines; summer-collected: open squares, dashed lines. Data are offset on the x-axis to better show means and variance. Each point is the population mean JGR on each diet with 1 standard error using genotype as the level of replication.

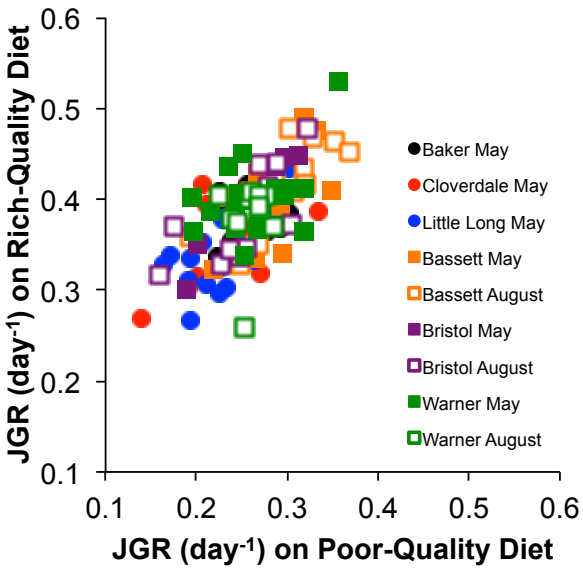

Figure 1.2. Growth rates on the two algal diets. *Ankistrodesmus* (rich-quality diet) JGR and *Oocystis* (poor-quality diet) JGR are positively correlated (Pearson Correlation Coefficient = 0.657, $P < 0.0001$). Genotypes from the two persistence types are differentiated by shape (non-persisting: circles; persisting: squares). Populations are differentiated by color. In the persisting populations, season of collection are differentiated by open (spring) or closed (summer) squares.

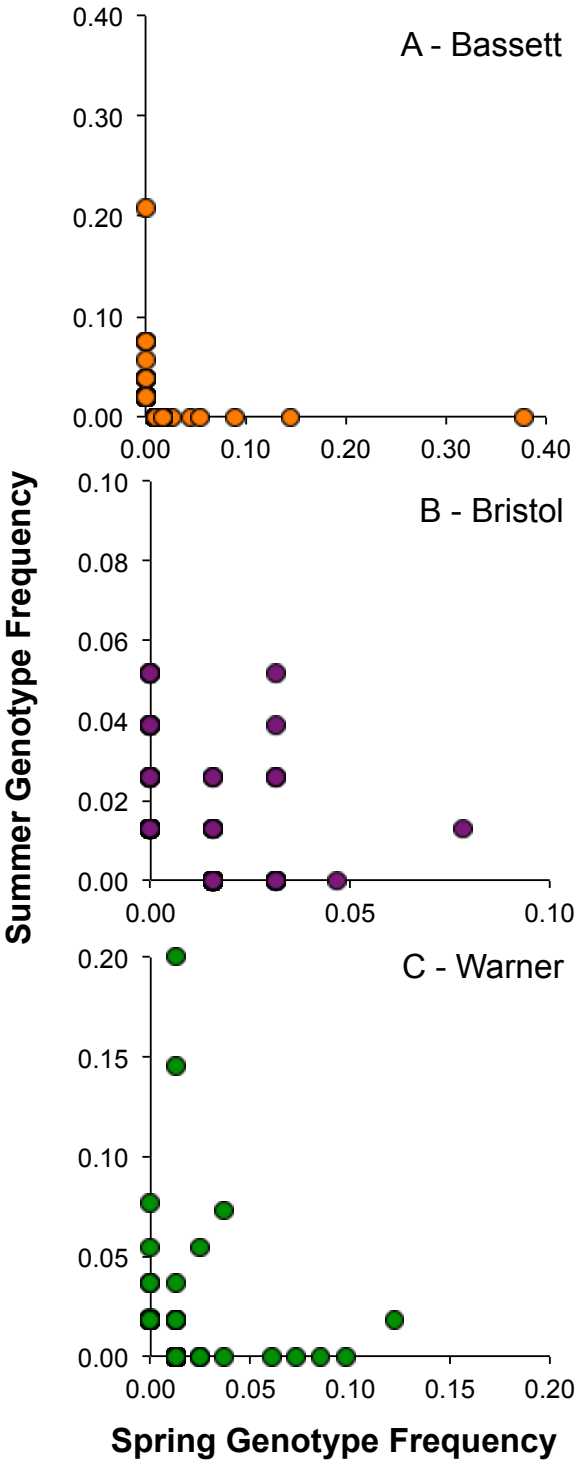

Figure 1.3. Frequencies of genotypes collected from the three persisting populations (A = Bassett Lake; B = Bristol Lake; C = Warner Lake) in spring (May) 2012 and summer (August) 2012. Each point is a unique genotype. Note that in Bassett Lake (A) there is no overlap between the clones collected in the spring and in the summer.

Chapter 2: Grow big or go home: *Daphnia pulicaria* clones with higher growth rates and from more stable populations are less likely to invest in sexually-produced dormant offspring.

Abstract

Environmental heterogeneity often results in periods of low fitness. Two common strategies for coping with this heterogeneity are dormancy and sexual reproduction. Dormancy is a strategy of temporal escape from poor conditions and sexual reproduction may result in increased fitness for progeny. Here, we investigate why there is significant intraspecific variation in the likelihood of investing in sexually-produced dormant offspring both between and within populations of the facultative parthenogen *Daphnia pulicaria*. Due to physiological mechanisms, investment in dormancy is synonymous with investment in sexual reproduction in this organism. Using 172 genotypes from six populations, we investigated potential mechanisms for the maintenance of this variation in sex/dormancy investment. We found that individuals from two out of our three populations with high fluctuation in annual density were more likely to invest in sex/dormancy. At the individual level and using growth rate as a proxy for active-stage fitness, we supported our prediction that individuals with higher fitness were less likely to invest in sexually-produced dormant offspring. Individuals with lower active-stage fitness were more likely to invest in sexually-produced dormant offspring, although variation suggests other causal factors determine a genotype's likelihood of investing in sex/dormancy. Most individuals that invested in sex/dormancy were specialists for one of the two modes of reproduction: production of either clonal males or haploid eggs. This study demonstrates that there is a relationship between growth rate (a proxy for expected fitness in the active stage for a *Daphnia*) and the likelihood of investing in sexually-produced dormant offspring. We further discuss these results in context with other factors that influence a genotype's likelihood of investing in sex/dormancy, such as response to predation and parasitism.

Introduction

Variation in the fitness of individuals is common within species and populations (Bolnick et al. 2003, Bolnick et al. 2011, Violle et al. 2012). Coupled with substantial environmental variation between habitats and seasons, this means that individuals may respond differently to regular changes in environmental quality (Chesson and Huntly 1997). Furthermore, individuals may differ in their response to environmental heterogeneity by investing in sexual reproduction and/or dormancy (Kirk 1997, Tessier et al. 2000, Reznick et al. 2000, Tessier and Cáceres 2004, Otto 2008, D'Souza and Michiels 2010). An individual's response to environmental heterogeneity may explain why some species maintain intraspecific variation in likelihood of investing in sexual reproduction and/or dormancy. Investment in sexual reproduction and dormancy both come with inherent reproductive costs and are commonly viewed as a cost-benefit analysis of reduced immediate fitness for the potential of future fitness gains (Lynch 1983). Therefore, individuals with low active stage fitness (the fitness of individuals who are not dormant) may be the most likely to invest in sexual reproduction and/or dormancy. In this study, we use a facultatively parthenogenetic species that can create dormant progeny to investigate how individual differences in active-stage fitness (as measured by juvenile growth) influence the likelihood of investing in sexual reproduction and dormant offspring.

Sexual reproduction typically results in offspring that are genetically different from the parents and has both costs and benefits for facultative parthenogens, individuals that can switch between asexual and sexual reproduction (Mort 1991, Rispe et al. 1998, Simon et al. 1999, Innes and Singleton 2000, Dedryver et al. 2001). Theory predicts that individuals that are in poorer habitat conditions or who have low fitness are more likely to invest in sexual reproduction (Redfield 1988, Hadany and Otto 2009). While investing in sexually-produced offspring is not a guarantee that the progeny will have a higher fitness than parents, there is a possibility that some sexually-produced offspring will have a phenotype with high fitness (Jaenike 1978, Hamilton 1980, Lloyd 1980, Hamilton et al. 1990, Lande and Shannon 1996, Otto 2008, Salathé et al. 2009, Simberloff 2009, Lively 2010, Starrfelt and Kokko 2012, Scheiner 2014). Investing in sexual reproduction can be risky, as individuals must find a mate, successfully produce offspring, and only half of the genome is passed on to offspring (Otto and Lenormand 2002). Additionally, in facultative parthenogens, sexual reproduction reduces the potential energy allocation to current growth and asexual reproduction (Ellner 1997, Otto and Lenormand 2002, Shefferson et

al. 2003). Therefore, investment in sexual reproduction by facultative parthenogens is predicted to be low, with individuals primarily reproducing clonally, unless there is high environmental variability or individual fitness is low.

Dormancy is a common strategy against seasonal environmental heterogeneity as it allows individuals, eggs, or seeds to disperse temporally and potentially survive detrimental environmental conditions before repopulating a habitat (Cohen 1966, Mort 1991, Stearns 1992, Hairston et al. 1995, Cáceres 1997, Cáceres 1998, Burt 2000, Innes and Singleton 2000, Dedryver et al. 2001, Huxman et al. 2013, Gremer and Venable 2014). However, investing in dormancy also has costs and can therefore be viewed as an evolutionary cost-benefit analysis. Theory suggests that dormancy should only occur when the active-stage fitness of an individual declines below the potential fitness in the dormant stage; when fitness is higher in the active stage, non-dormant offspring are favored (Cohen 1970, Taylor 1980). In some species, this is in part due to the energetic costs of producing dormant offspring (McCauley et al. 1999). Dormant individuals are not immune to risk and are still vulnerable to threats such as predation (Cáceres and Hairston 1998), failing to receive the necessary hatch cues, or hatching at inopportune times (Cáceres and Tessier 2003). Allocation to dormancy is predicted to be low for an individual living in a stable environment with low threat; however when habitat variability increases, so too does the likelihood of individuals investing in dormancy (Stearns 1992, Cáceres 1997, Reznick et al. 2000, Walsh 2013, Gremer and Venable 2014).

Studies of facultative sexuality and likelihood of investing in dormancy are common in zooplankton (Hairston and Munns 1984, De Stasio 1989, Hairston 1996, Cáceres 1998, Brendonck and De Meester 2003, Cáceres and Tessier 2003, Cáceres and Tessier 2004b, Fitzsimmons and Innes 2006, Radzikowski 2013). Additionally, prior research has shown that there is often intraspecific variation in the likelihood of investing in sexual reproduction and dormancy (Yampolsky 1992, Deng 1996, Innes and Singleton 2000). For species such as *Daphnia pulicaria*, it is impossible to disentangle whether an individual is investing in sexual reproduction or dormant offspring as both processes are mechanistically linked (Bonner 1958, Bell 1982, Mort 1991, Tessier and Cáceres 2004a, Decaestecker et al. 2009). Therefore, the costs and benefits of allocation to sexual reproduction must be considered alongside the costs and benefits of allocation to dormant offspring when considering why some individuals are more likely to invest in sexual reproduction and/or dormancy (Bonner 1958, Simon et al. 2002, Serra

et al. 2005). In this study, we do not focus on the evolution or maintenance of sexual reproduction in facultatively parthenogenetic species; instead we focus on factors that may influence an individual's likelihood of investing in sexually-produced dormant offspring.

We build on prior research that has demonstrated intraspecific variation in sex/dormancy investment both between and within populations of *Daphnia pulicaria* in central Michigan. In the populations used in this study, allocation to sex/dormancy occurs only during a short period of the spring in which clonal males are produced and mature before females create haploid eggs that, if fertilized, will be obligately dormant (Cáceres and Tessier 2004a). Cáceres and Tessier (2004a) and Tessier and Cáceres (2004) found that investment in sex/dormancy was the highest in populations that have a greater annual decline in density. Some of these populations with high annual density fluctuation (here termed "non-persisting populations") are reduced to undetectable levels by the summer due to combined effects of increased predation, parasitism, and reduced resource quality common in freshwater lakes (Sommer et al. 1986, Tessier and Welser 1991, Lampert and Sommer 1997, Ebert et al. 1997, Cáceres and Tessier 2004a, Cáceres and Tessier 2004b, Johnson et al. 2006a, Duffy et al. 2010, Sommer et al. 2012). Over a decade after the sampling of Cáceres and Tessier (2004a) and Tessier and Cáceres (2004), the population density of *D. pulicaria* still declines to undetectable levels in the summer in these non-persisting populations; therefore, it is likely individuals in these non-persisting populations are still heavily investing in sexually-produced dormant offspring during the spring.

Within populations, Tessier and Cáceres (2004) demonstrated that individual *Daphnia pulicaria* genotypes differ in their likelihood of investing in sexually-produced dormant offspring. It is possible that there are individual differences that are driving this variation in allocation. For example, if an individual has low active-stage fitness or anticipates low fitness when conditions deteriorate in the summer, that genotype may be more likely to invest in sexually-produced dormant offspring for the benefits of sexual reproduction and/or dormancy (Cohen 1966, Redfield 1988, Chesson and Huntly 1997, Otto 2009). Additionally, Tessier and Cáceres (2004) demonstrated that some of these genotypes were specialists who produced exclusively males or unfertilized haploid eggs. Individuals who are specialists for the production of only males or only sexual females (the haploid eggs in the ephippia) are relatively common across species of *Daphnia.* Studies in both *D. pulex* and *D. magna* have demonstrated a genetic predisposition of allocating to either males or sexual females (Innes 1997, Galimov et al. 2011).

Due to the high chance of inbreeding during the short window of sexual reproduction that many *Daphnia* populations experience (including the populations studied here), De Meester and Vanoverbeke (1999) and Cáceres et al. (2009) posited that the specialization of clones to either produce males or sexual eggs reduced the likelihood of inbreeding. Further differentiation of specialists for one of the two sexes occurs as a result of some *Daphnia* genotypes being non-male investing clones, those who allocate only to asexual daughters or haploid eggs for fertilization by males (Innes and Dunbrack 1993, Galimov et al. 2011). Not exclusive to *Daphnia*, specialization for one or the other sex also occurs in aphids (Rispe et al. 1999).

Intraspecific variation in the likelihood of investing in sexual reproduction and dormancy has a genetic component in *Daphnia* with some genotypes more likely to invest in sex/dormancy than others (Yampolsky 1992, Deng 1996, Innes 1997, Tessier and Cáceres 2004). Here we ask if this intraspecific variation in sex/dormancy investment is linked to a proxy for the fitness of an individual in the active-stage: juvenile growth rate. In *Daphnia*, juvenile growth is a correlate of expected fitness for active stage individuals (Lampert and Trubetskova 1996, Tessier et al. 2000, Hairston et al. 2001, Crawford et al. 2015). Genotypes may differ in their mean response to resource quality (Kassen 2002) and this variation has been extensively studied in *Daphnia* through differences in capture of resources and how those resources are allocated (Threlkeld 1979, Weider 1985, Leibold 1991, Spitze 1991, De Meester et al. 1995, Kirk 1997, Reznick et al. 2000, Tessier et al. 2000, Tessier and Woodruff 2002, Jeyasingh et al. 2003, Boon et al. 2007, Allen et al. 2010, Brzeziński et al. 2010, Hall et al. 2012, Glücksman et al. 2010). In a previous study (Chapter 1), we assayed the growth rate of many of the same individuals used in this study. Here, we ask if intraspecific variation in growth (a correlate of active-stage fitness) is related to intraspecific variation in the likelihood of investing in sexually-produced dormant offspring.

Given intraspecific variation in the likelihood of *Daphnia pulicaria* genotypes to invest in sex/dormancy (Cáceres and Tessier 2004a, Tessier and Cáceres 2004), we examined potential mechanisms to explain why this variation exists. In this study, we quantified the investment in sex/dormancy of individuals from each of six populations. We predicted that populations with greater variation in annual density would invest in more sexually-produced dormant offspring, potentially as a temporal escape from the poor habitat quality of the summer. To determine if allocation to sex/dormancy has been maintained in the non-persisting populations, we compared our laboratory assay of likelihood of investing in sex/dormancy to the field data collected by

Cáceres and Tessier (2004a). At the individual level, we predicted that sex/dormancy investment would be inversely correlated with an individual's expected fitness in the active stage (as measured by juvenile growth rate). As there are fitness costs to a genotype reproducing sexually or producing dormant offspring, we predicted that individuals with high mean growth rates would invest little in sex/dormancy. Conversely, individuals with low expected fitness in the active stage were predicted to be more likely to gain the benefits of sexual reproduction and/or dormancy. As a result of the reduced likelihood of inbreeding depression, we predicted that the majority of individuals investing in sexually-produced dormant offspring would be specialists and produce clonal daughters and either clonal sons or haploid eggs.

Methods

Field Populations and Genetic Analysis

To test our questions regarding investment in sexual reproduction and dormancy, we worked with genotypes collected from populations of *Daphnia pulicaria* in six lakes in central Michigan near the Kellogg Biological Station (Barry Co. and Kalamazoo Co., MI, USA). Prior studies indicate that population density peaks in the spring but the magnitude of decline in *D. pulicaria* density differs between lakes. Therefore, some populations do not persist at detectable densities throughout the year (Tessier and Welser 1991, Geedey et al. 1996, Cáceres and Tessier 2004a, Tessier and Cáceres 2004b, Duffy et al. 2009). We chose three lakes with standing *D. pulicaria* populations throughout the year ("persisting" – Bassett, Bristol, and Warner) and three that reached undetectably low densities by the summer ("non-persisting" – Baker, Cloverdale, and Little Long). To conduct our assays, we collected and isolated >100 live individuals from each population in May of 2012 and 2013. In the three persisting populations, we also collected individuals in August of 2011 and 2012. To ensure that assayed clonal lines were unique from all others from the same population at the same time of collection, we genotyped at six microsatellite loci (Dp 27, 78, 102, 196, 433, 461; Colbourne et al. 2004, Cristescu et al. 2006, Allen et al. 2010, Holmes et al. 2016, Chapter 1).

Laboratory Assays

We used laboratory life-table assays of 172 genotypes of field-collected *Daphnia pulicaria* to test our predictions that population type (persisting vs. non-persisting populations), population of collection, and season of collection may influence investment in sexual reproduction and/or dormancy. First was a period of standardization of maternal effects for three generations according to Lynch and Walsh (1998). We modified the life-table for allocation to sexual reproduction used by Cáceres and Tessier (2004a) and Tessier and Cáceres (2004). Individual female neonates were isolated in 110mls of filtered lake water. *Daphnia* individuals were raised in an environmental chamber at 20°C and a spring photoperiod of 14[light]:10[dark]. Using high-density batch cultures (as in Hobaek and Larsson 1990, Stelzer and Snell 2003, Tessier and Cáceres 2004), we created kairomone-dense water that gives the *Daphnia* the chemical signatures of a high density *Daphnia* population while allowing us to keep individuals isolated. An 80:20 mixture of filtered lake water and filtered mass culture (kairomone) water was added to each beaker and changed three times per week. Immediately after the water change, 2mg dry weight/L of the green algae (*Ankistrodesmus falcatus*) was added to each beaker. We used a spring photoperiod, temperature, density, and resource combination because *Daphnia pulicaria* from these populations only invest in sex/dormancy during a short period of the spring (Cáceres and Tessier 2004a).

Upon maturation, individuals were observed three times per week for production of clutches of clonal daughters, clonal sons, or ephippia. The ephippium is a modification of the carapace that encases two haploid eggs waiting for fertilization by a male (Mort 1991, Decaestecker et al. 2009). If fertilized, these eggs undergo a period of obligate dormancy prior to hatching (Cáceres 1998). For each asexual clutch, offspring were removed from the beaker, counted, and identified as male or female under a dissection microscope. In the facultatively sexual *Daphnia pulicaria*, male progeny are important metrics of investment in sex/dormancy as they are necessary for the fertilization of the eggs in the ephippia that will undergo obligate dormancy. This process continued until individuals had produced their sixth clutch. Some individuals died prior to reaching the sixth clutch, only those that released their third clutch and subsequently died were included in the analysis. Each genotype had multiple clonal individuals as replicate measures; when we pooled the reproductive events per genotype there was an average of 22.5±0.4 clutches per genotype with a minimum of 8 and a maximum of 38 clutches.

We used the results of a prior assay (Chapter 1) on 121 of the same clones to determine if there is a negative relationship between overall growth rate and sex/dormancy investment. The juvenile growth rate assay is a measure of increase in somatic tissue during the juvenile stage and the results are commonly used as a correlate of per capita growth and fitness during the active stage in *Daphnia* (see Tessier and Goulden 1987, Lampert and Trubetskova 1996, Desmarais and Tessier 1999, Tessier et al. 2000, Tessier and Woodruff 2002, Hairston et al. 2001, Crawford et al. 2015). We grew individuals on two algal diets from which we calculated each genotype's mean growth rate (Chapter 1). We used mean growth rate as a measure of a genotype's overall performance as results from Chapter 1 determined that a genotype's growth on each of the two algal diets was positively correlated (Pearson Correlation Coefficient = 0.657, $P < 0.0001$).

Analyses

Ephippial investment was calculated as the proportion of total clutches in which females formed and shed an ephippium. Given that we encounter both all-male and mixed-male/female clutches, male investment was calculated as the proportion of males to total offspring produced. Sum investment in sexually-produced dormant offspring was standardized at the clutch-level as the proportion of reproductive events that were ephippial clutches, male-only clutches, and mixed male/female clutches (which were counted as half). In all analyses we chose to include all genotypes including those with zero investment in sexual/dormant clutches, as there is biological importance to having individuals that do not allocate to sexual reproduction or dormancy despite being presented with conditions that would typically induce this allocation in *D. pulicaria* in nature.

To test whether individuals from different population types (persisting vs. non-persisting) or populations of collection grouped together, we used a multiresponse permutation procedure (MRPP) (Talbert and Cade 2013; R package Blossom, R version 3.1.3, R Core Team 2013). MRPP is used to compare the intragroup average distances of groups of data to determine if individuals from a group are more likely to be close together (Cáceres and Tessier 2004a; Talbert and Cade 2013). For this analysis, we ran separate models to determine the effect of the grouping of type (persisting vs. non-persisting), population of collection, and season of collection. A significant effect in the MRPP analysis suggests that individuals within a group are more similar to each other than to individuals of another group. As season of collection did not differ in

grouping (Standardized Test Statistic = 0.709, N = 77, $P = 0.744$), we combined May- and August-collected clones for all analyses of the relationship between growth and sex/dormancy investment.

We further assessed the likelihood of type- and population-level investment in sexually-produced dormant offspring through sets of Nested ANOVAs (SAS, version 9.4, Proc GLM). Our first set of ANOVA models focused on May-collected individuals from both persisting and non-persisting populations and tested for the effect of population type (persisting vs. non-persisting) and population of collection (nested in type) as fixed factors. We combined collection years, as there was no difference in investment across the years of collection (ANOVA $F_{2,496} = 1.81$, $P = 0.165$). Our second set of ANOVA models focused on the three persisting populations and assessed a difference between May-collected and August-collected individuals with population of collection and season of collection (nested in population) as fixed factors. For both sets of models, we tested the effects on three response variables each: sum dormant/sexual clutches, proportion ephippia clutches, proportion of males produced.

To determine if the likelihood of investing in sexually-produced dormant offspring was maintained, we compared our laboratory results to data collected between 1999 and 2001 by Cáceres and Tessier (2004a). Cáceres and Tessier (2004a) searched samples for females carrying ephippial eggs or males and calculated the proportion of each of these metrics of investment in sex/dormancy. We assessed the decadal correlation in investment in sex/dormancy via Pearson Correlation using the means of each population's sum, ephippial, and male investment.

Our prediction that there would be a negative relationship between sexually-produced dormant offspring and mean growth rate was tested using Quantile Regression (SAS, version 9.4, Proc QUANTREQ), due to the wedge-shape relationship between investment in sex/dormancy and mean growth. The Quantile Regression is robust against heterogeneous variance in the response variable (Koenker and Bassett 1978, Koenker & Machado 1999, Cade and Noon 2003). As ecologists cannot always measure all predictive variables, Quantile Regressions are useful in determining a relationship between two variables when there may be more hidden variables influencing a positive or negative linear relationship (Rosenbaum 1995, Cade and Guo 2000, Dunham et al. 2002, Cade and Noon 2003). This analysis generates regression lines through the quantiles of the data and a slope significantly different from zero indicates that there is a trend in the data similar to the use of standard linear regression (Cade and Noon 2003, Gotelli and Ellison

2004). Here, we used the 90th and 50th quantiles to test for a negative relationship between mean growth and sex/dormancy investment. The 50th quantile is the median for the data and is identical to the least absolute deviation (LAD) (Cade et al. 1999). The 90th quantile represents a maximum rate of change that may better explain the overall trend of the data that, due to other unmeasured dependent variables, would be obscured in a standard linear regression of the mean (Cade et al. 1999, Koenker and Hallock 2001, Cade and Noon 2003).

We predicted that genotypes would be specialists for production of either males or ephippia, not generalists investing in both types of sexually-produced dormant offspring. We used the Chi-Squared Goodness of Fit Test (R, Version 3.1.3, R Core Team 2013) to determine if there were a higher-than-random proportion of genotypes that were specialists, generalists, or not investing in sex/dormancy. Our null model was that a genotype would be equally likely to not invest in sex/dormancy, be specialists and produce exclusively males or ephippia, or be generalists and have a mixed allocation to sexual reproduction. Prior results by Innes (1997) in *Daphnia pulex* and Galimov et al. (2011) in *Daphnia magna* found that females generally allocate ≤40% of their offspring as males and ephippia production was typically slightly lower. This is coupled with sex ratio theory that suggests that mean production of each sex should be equivalent at the population level (Fisher 1930, Charnov 1982). Theory therefore suggests that of the roughly two thirds of individuals investing in sex/dormancy, roughly 50% should allocate to males and 50% should allocate to haploid eggs. We therefore set the hypothesized proportions equal to determine if the actual proportions of specialists, generalists, or non-investing genotypes differed from this null distribution. If proportions differ, this indicates that a greater proportion of genotypes are specialists or generalists than a null distribution.

Results

Populations differed in mean likelihood of investing in sexually-produced dormant offspring with two of the three non-persisting populations investing substantially in sex/dormancy (Fig. 2.1A-C; Table 2.1). This population-level variation existed between the May-collected individuals from the six populations and was largely driven by the high investment in all three metrics of sex/dormancy by individuals collected from Cloverdale and Little Long lakes (Table 2.1). Populations differed in investment in males with the highest mean

male investment in Little Long (Fig. 2.1B; Table 2.1). Populations also differed in investment in ephippial clutches with the greatest investment in Cloverdale (Fig. 2.1C; Table 2.1). Although there was no difference in investment in sex/dormancy based on type (persisting vs. non-persisting populations), two of the non-persisting populations (Cloverdale and Little Long) consistently had the highest mean investment in sex/dormancy (Fig. 2.1; Table 2.1). One non-persisting population's (Baker) low mean investment in sex/dormancy contributed to the lack of difference between persisting and non-persisting population types (Fig. 2.1; Table 2.1). Additionally, despite Cloverdale and Little Long's high mean investment, there were still individuals collected from those populations that did not allocate to sex/dormancy in the laboratory assays.

Our results on the overall investment in sexually-produced dormant offspring are consistent with the field data collected on these six populations by Cáceres and Tessier (2004a) (Fig. 2.1). We found a positive correlation in investment in sex/dormancy (Fig. 2.1A; Pearson Correlation Coefficient = 0.962, $P = 0.002$) and male investment (Fig. 2.1B; Pearson Correlation Coefficient = 0.842, $P = 0.035$) between our laboratory-based assays on spring-proxy conditions and the field-collected data from Cáceres and Tessier (2004a). Ephippial investment was the only area where the laboratory metrics differed from the field metrics of Cáceres and Tessier (2004a); Little Long individuals invested far less in ephippia in the lab conditions than they did in the field (Fig. 2.1C; Pearson Correlation Coefficient = 0.556, $P = 0.252$).

Individuals collected in the spring and summer did not differ in their likelihood of investing in sexually-produced dormant offspring in the laboratory (Fig. D.1; Table D.1). These results suggest that, although a short period of time during the spring is the only time in which individuals invest in sex/dormancy in the field, individuals active during the summer are equally capable of engaging in sexual reproduction and production of dormant offspring if given the cues of spring conditions. Results from considering ephippial production and male production independently also yielded no difference in investment between the three persisting populations or between the two seasons of collection (Fig. D.1; Table D.1).

There was among-population variation in investment in sexually-produced dormant offspring and we supported our prediction that individuals from the same type (persisting vs. non-persisting) and population of collection would be more likely to group together (Fig. 2.2). Population types group together, with non-persisting populations grouping at a lower mean

juvenile growth rate (JGR) and higher mean investment in sex/dormancy compared to persisting populations (Standardized Test Statistic = -27.391, N = 121, $P < 0.0001$; Fig. 2.2A). This type-level grouping is equivalent for investment in males (Standardized Test Statistic = -14.04, N = 121, $P < 0.0001$; Fig. 2.2B) and investment in ephippia (Standardized Test Statistic = -17.88, N = 121, $P < 0.0001$; Fig. 2.2C). We also found a significant grouping effect of population on sum investment in sex/dormancy (Standardized Test Statistic = -16.21, N = 121, $P < 0.0001$; Fig. 2.2A), investment in males (Standardized Test Statistic = -14.40, N = 121, $P < 0.0001$; Fig. 2.2B), and investment in ephippia (Standardized Test Statistic = -21.63, N = 121, $P < 0.0001$; Fig. 2.2C). In the case of populations, individuals from Cloverdale and Little Long group together with lower mean JGR and higher mean investment in sex/dormancy while individuals from the other four populations group with low mean sex/dormancy investment and higher mean JGR. Bassett has the lowest mean investment in sex/dormancy and the highest mean JGR. We found no grouping effect of season (Standardized Test Statistic = 0.709, N = 77, $P = 0.744$) and therefore include individuals collected in both spring and summer from each of these populations.

At the individual-level, we found that individuals with higher mean juvenile growth rate (JGR) were less likely to invest in overall production of sexually-produced dormant offspring (Fig. 2.3). Using quantile regression on the sum investment in sex/dormancy, we found a negative relationship at the 90th quantile (b_1 = -2.41, 95% Confidence Interval = -4.35 – -0.48, $P = 0.015$) and at the 50th quantile (b_1 = -0.64, 95% Confidence Interval = -0.27 – -1.18, $P = 0.022$) (Fig. 2.3A). These results show that the slope (b_1) is negative and significantly differs from a slope of zero, although there is still variation. There was no difference in slope for the other two metrics of sex/dormancy (males and ephippia production); therefore, there was no relationship between male or ephippial investment and mean JGR. For male investment, using quantile regression we found no relationship at the 90th quantile (b_1 = -1.38, 95% Confidence Interval = -3.49 – 0.75, $P = 0.200$) or at the 50th quantile (b_1 = -0.36, 95% Confidence Interval = -0.75 – 0.04, $P = 0.077$) (Fig. 2.3B). Ephippial investment yielded similar quantile regression results with no relationship at the 90th quantile (b_1 = -0.71, 95% Confidence Interval = -2.04 – 0.62, P = 0.29) or at the 50th quantile (b_1 = 0, 95% Confidence Interval = 0) (Fig. 2.3C). Therefore, the negative relationship that we observed between sum investment in sex/dormancy and mean JGR does not extend to investment in males or investment in ephippia.

One reason for the lack of relationship between mean juvenile growth rate (JGR) and an individual's likelihood of investing in males or ephippia may be that individuals with low JGR were more likely to be generalists and invest in both male and ephippial offspring as well as asexual daughters. As in Tessier and Cáceres (2004), many genotypes were specialists and produced either males or ephippia, with relatively few genotypes investing in both types of sexual/dormant progeny (Fig. 2.4). Of the 172 assayed genotypes, we found that generalist genotypes investing in all modes of reproduction (production of males, mixed clutches, ephippia, and asexual daughters) only comprised 16.9%. Despite this low percentage, generalist genotypes with low mean JGR (JGR < 0.32) often had high investment in both males and ephippia including several individuals with six out of 11 generalist genotypes investing over 40% in sexual/dormant offspring. However, of the 10 generalist genotypes at high mean JGR (JGR > 0.32), none invested more than 33% in sexual/dormant offspring. Therefore, these low-growing generalist individuals with high investment in sex/dormancy contributed to the relationship between JGR and sum investment in sexually-produced dormant offspring (Fig. 2.3A) but did not influence the relationship between male (Fig. 2.3B) or ephippial investment (Fig. 2.3C) and JGR. Most genotypes were specialists (47.7%), with 18.0% exclusively producing either ephippia or female-only clutches and 29.7% producing male-only, mixed male-female, and female-only clutches. The final 35.5% of the genotypes never invested in sexually-produced dormant offspring in the laboratory assay.

The distributions of specialist and generalist for investment in sexually-produced dormant offspring differ from a null distribution that a genotype would be equally likely to not invest in sex/dormancy, be a specialist for males or ephippia, or be a generalist and invest in all forms of reproduction (Chi-Squared Goodness of Fit = 14.42, df = 2, $P < 0.0001$). Using MRPP analysis, we found that populations group together (Standardized Test Statistic = -30.46, N = 172, $P < 0.0001$). This grouping is largely driven by Little Long's low overall investment in ephippia but higher investment in males and Cloverdale's higher ephippia investment and, with the exception of one individual, low investment in males (Fig. 2.4).

Discussion

We examined possible explanations for intraspecific variation in investment in sexual reproduction and dormancy within and among six populations of *Daphnia pulicaria* in Michigan. Our findings support prior results and hypotheses that individuals are more likely to invest in sex/dormancy when they are in riskier habitats or if they have a lower expected fitness (Hairston and Munns 1984, Hairston and Van Brunt 1994, De Stasio 1998, Pigliucci and Schlichting 1998, Gessler and Xu 2000, Hadany and Beker 2003, Hadany and Otto 2007, Otto 2008, Otto 2009, Smith and Snell 2012, Walsh 2013). *Daphnia* from two of the three higher-risk (non-persisting) populations were more likely to invest in sexual reproduction and dormancy. Individuals with higher mean juvenile growth rate, a correlate of fitness in the active stage, were less likely to invest in sexually-produced dormant offspring. We also found that many genotypes were specialists who invested only in one form of sexual reproduction while generalist individuals generally had low mean juvenile growth rate. Together, our results suggest that individuals at greater risk are more likely to invest in sexual reproduction and dormant offspring, possibly to gain the benefits of sexual reproduction, temporal escape for offspring through dormancy, or the joint effects of sexual reproduction and dormancy.

Dormancy investment is common when cumulative conditions reduce the fitness of active individuals (Pigliucci and Schlichting 1998, Walsh 2013) and is especially common when individuals have reliable cues for the onset of poor conditions (Hairston and Munns 1984, Hairston and Van Brunt 1994). Our study supports the results of Cáceres and Tessier (2004a) who found that *Daphnia pulicaria* individuals are more likely to invest in sex/dormancy if the population is at a greater risk of decline in density. We go further in this study by demonstrating that, although Cáceres and Tessier (2004a) showed that dormancy investment in the field only occurs during a short period of the spring, summer-collected genotypes are equally capable of investing in sex/dormancy as their spring-collected counterparts when given the same cues. This implies that genotypes can invest in dormancy during other times of the year but do not; instead they most likely hone in on the density, photoperiod, temperature, and resource cues of the spring for the induction of sexual reproduction and production of dormant offspring (Hebert 1978, Carvalho and Hughes 1983, Hobaek and Larsson 1990, Kleiven et al. 1992, Innes and Singleton 2000, Spaak 1995, Deng 1996). Only individuals collected from Little Long Lake responded differently in the laboratory conditions with our lab-reared clones producing fewer

ephippia than the field population does in the spring (Cáceres and Tessier 2004a). Tessier and Cáceres (2004) also found that individuals from Little Long Lake produced no ephippia under similar laboratory conditions. Despite these populations investing in sexually-produced dormant offspring in the field, they do not appear to respond to the cues we have presented in the lab.

Dormancy in temporally heterogeneous environments may help to buffer genotypes from extinction when there are annual fluctuations in habitat quality. A population-type propensity to invest in dormancy has been shown previously in *Daphnia magna* in which individuals from ephemeral ponds were more likely to invest in production of dormant offspring than those from permanent water bodies (Lynch 1983, Yampolsky 1992, Roulin et al. 2013). Results from Cáceres and Tessier (2004a) and this study also demonstrate that there is intraspecific variation in dormancy investment within populations living in permanent lakes. The non-persisting populations of *Daphnia pulicaria* experience an intense combination of predation, parasitism, and competition for resources that ends up driving down the *D. pulicaria* populations to nearly undetectable levels in the summer (Cáceres and Tessier 2004a). Due to the reduction in population density, our results align with a modeling study by Vitalis et al. (2013), who found that local extinctions are likely to select for an increase in dormancy investment. Additional empirical work by Smith and Snell (2012) demonstrated that rotifers can rapidly invest in dormancy in heterogeneous environments, allowing individuals to invest in offspring that are preserved for the future and the possibility that environmental conditions will improve. Furthermore, a review by de Casas et al. (2015) found that dormancy is favored when habitats experience regular risk resulting in a significant reduction of population abundance. In sum, our results align with prior studies and demonstrate that dormancy allocation is generally higher in populations with greater variation in habitat quality.

We must also consider the benefits of sexual reproduction as, in *Daphnia pulicaria*, investment in dormant offspring cannot be disentangled from investment in sexual reproduction. It is therefore possible that individuals are investing in sexual reproduction in addition to, or instead of, investing in dormancy. The benefits of occasional sexual reproduction have been demonstrated in facultative parthenogens that predominantly reproduce clonally unless conditions deteriorate. Delmotte et al. (2002) and Guillemaud et al. (2003) both demonstrated that aphids invest more in sexual reproduction when in temporally heterogeneous environments. Occasional sexual reproduction in facultatively parthenogenetic species has many benefits

(reviewed in D'Souza and Michiels 2010). For example, Peck (1993) posited that rare sex might increase the rate by which beneficial mutations are fixed in a population. In species that predominantly reproduce clonally, even rare investment in sexual reproduction can increase genotypic diversity, which has the possibility of increasing phenotypic diversity (Balloux et al. 2003, Bengtsson 2003, Allen and Lynch 2012) and even the rate of phenotypic evolution (Lynch and Gabriel 1983).

Fitness-associated sex is one of the potential explanations of why some individuals are investing more in sexually-produced offspring (Redfield 1988, Kleiven et al. 1992, Agrawal et al. 2005, Otto 2009, Griffiths and Bonser 2013). Redfield (1988) posited that organisms are more likely to allocate to sex when the individual has low current fitness. Hadany and Otto (2009) supported this hypothesis through a model and demonstrated that individuals in poor habitat conditions should reproduce sexually while those in good habitat conditions should maintain asexual reproduction. In this study, we linked the intraspecific variation in growth with likelihood of investing in sexually-produced dormant offspring. Prior results have demonstrated that individuals differ in their ability to grow on available resources (Tessier et al. 2000, Jeyasingh et al. 2003, Glucksman et al. 2010, Hall et al. 2012, Chapter 1). Results from our quantile regression analysis indicate that there is a negative relationship with high-growing genotypes investing less in sex/dormancy than the lower-growing genotypes. While variation did exist with some low-growing genotypes not investing in sexual reproduction, some low-growing individuals allocated up to 40% of reproductive investment towards ephippia and others invested up to 70% in males. Some low-growing genotypes were generalists and invested in both ephippia and males, further reinforcing the negative relationship in sum investment in sexually-produced dormant offspring.

We also found type- and population-level groups in the negative relationship between juvenile growth and sex/dormancy investment in which individuals from populations with low mean growth were more likely to invest in sex/dormancy. The type-level (persisting vs. non-persisting) grouping was driven by Cloverdale and Little Long's low growth rate and high sex/dormancy investment. Although our ANOVA analysis showed no difference between population types in their mean investment in sexually-produced dormant offspring, the MRPP analysis of groups demonstrate that non-persisting populations group together with lower mean JGR and higher mean investment in sex/dormancy.

The high demands of sexual reproduction likely play a role in the short window of sex/dormancy allocation observed in these populations of *Daphnia pulicaria* and may also influence why many genotypes are specialists for one form of investment in sex/dormancy. A major limitation for sexual reproduction in facultatively parthenogenetic species is that timing of producing both sexes must align properly (Decaestecker et al. 2009). For successful sexual reproduction, *D. pulicaria* must have mature males in the water column at the time of production of the haploid eggs in the ephippia (Mort 1991). The short period of sexual reproduction in the field observed by Cáceres and Tessier (2004a) means that individuals receptive to reproducing sexually will invest in males prior to the investment in ephippia. We found that specialists made up nearly half of the genotypes assayed, including the 35.5% that did not allocate to sexual reproduction. Specialists were especially common in Cloverdale and Little Long, the two populations that invested the most in sexually-produced dormant offspring. Our results demonstrate that genotypes, especially those from high-sex/dormancy populations, are more likely to be specialists for two possible reasons. In this short period of reproduction in the field, there is a very high likelihood of inbreeding unless genotypes specialize with some investing primarily in males and others in ephippia. De Meester and Vanoverbeke (1999) and Cáceres et al. (2009) both posited that a benefit of specialist genotypes could reduce inbreeding depression. In populations that invest more in sex/dormancy, the non-persisting populations, segregation by specialization in production of males vs. haploids may reduce the likelihood of inbreeding. Alternately, Galimov et al. (2011) demonstrated that some *Daphnia* genotypes have genetically lost the ability to produce males; as a result, they suggest that genotypes have segregated with some specializing in male production while others specialize in the creation of haploid eggs.

Although we found that mean investment in sex/dormancy was higher in individuals with low mean growth rate, the wedge-shape of our data suggest that there are other causal factors influencing an individual's likelihood of investing in sex/dormancy. For instance, there were individuals with both low juvenile growth rate and low investment in sex/dormancy. We cannot measure all of the variables influencing the intraspecific variation in traits and there are likely other causal factors that should be included to better understand the intraspecific variation in investment in sex/dormancy (Rosenbaum 1995, Koenker and Hallock 2001, Cade and Noon 2003). Other factors such as resource heterogeneity and competition, predators, and/or parasites may influence the likelihood of individuals to invest in sexually-produced dormant offspring.

Juvenile growth rate has been commonly used as a proxy for expected fitness of active (non-dormant) *Daphnia* (Tessier and Goulden 1987, Lampert and Trubetskova 1996, Desmarais and Tessier 1999, Tessier et al. 2000, Hairston et al. 2001, Tessier and Woodruff 2002). However, just because an individual grows well on laboratory-raised proxy resources does not mean that it will have high fitness in the active stage in the field. For example, Crawford et al. (2015) found that *Daphnia ambigua* clones used in an experiment by Steiner et al. (2007) did not differ in juvenile growth rate despite these clones having competitive differences. Factors other than the mean juvenile growth rate of an individual can influence the fitness of that genotype in natural populations. Individuals may use also use sexual reproduction and dormancy as a means of predator or parasite resistance.

Predation is a factor that may influence the fitness of individuals in natural habitats that we do not examine in this study. Fish predators, especially young-of-the-year, exert a strong influence on *Daphnia* population density in the late spring and summer. The timing of sexual reproduction and investment in dormant progeny by *Daphnia pulicaria* precedes the increase in YOY fish predation in these populations. Therefore, dormancy may be employed in these populations as a means of temporal escape from high predation (Dzialowski et al. 2003, Mikulski and Pijanowska 2009, Slusarczyk et al. 2012, Walsh 2013). Predation by fish is especially high in the three non-persisting populations due either to a lack of deepwater refuge or because the refuge is anoxic (Gerrish and Cáceres 2003, Cáceres and Tessier 2004a). Walsh and Post (2012) found that predation could influence the likelihood of investing in sex/dormancy in populations of *D. ambigua*. In our study, *D. pulicaria* individuals collected from the high predation lakes had small body size, possibly to reduce visual predation risk (as reviewed in Hart and Bycheck 2011). As individuals from these populations are born at equivalent mass but mature at a smaller size (Chapter 1), these genotypes have lower mean juvenile growth rate than individuals who mature at a larger size. Therefore, individuals from Cloverdale and Little Long Lakes both mature smaller and invest comparatively more in sex/dormancy, which may combine to help them avoid predation by fish.

Sexual reproduction may also be a means of parasite resistance. An experimental study using snails by Jokela et al. (2009) found support for the prediction that common asexual genotypes are disproportionally affected by parasites. In daphniid populations, common clones are more likely to be parasitized than rare clones (Dybdahl and Lively 1998, Wolinska and Spaak

2009, Duffy et al. 2010). Prior studies by Hall et al. (2007, 2010, 2012) suggest that genotypes with higher growth rates also feed faster and are therefore more likely to be exposed to parasites. If these fast-feeding genotypes are more likely to be parasitized, there is a benefit of occasional sexual reproduction for parasite resistance. Duffy et al. (2010) demonstrated that parasites are highly host specific and species of parasites specifically target certain zooplankton species. Decaestecker et al. (2007) posited that parasites rapidly adapt to host genotypes, prompting a Red Queen dynamic in which host genotypes and parasites are constantly adapting. Sexual reproduction is one of the mechanisms by which *Daphnia magna* have been shown to rapidly adapt to coevolving parasites (Auld et al. 2016). This stress-induced allocation to sexual reproduction has been supported by recent experimental work; Hite et al. (*In Prep*) found that female *Daphnia dentifera* who were parasitized by the *Metschnikowia bicuspidata* fungus were more likely to invest in sexual reproduction, potentially as a means of increasing parasite resistance. Parasite resistance may therefore contribute to the variation we observed in the likelihood of individuals investing in sexually-produced dormant offspring. Future studies would benefit from including additional variables such as a genotype's response to predators and parasites, in conjunction with fitness analyses through growth, to better explain the intraspecific variation in likelihood of investing in sexually-produced dormant offspring through a multi-dimensional trade-off (as in Edwards et al. 2011).

For individuals that invest in dormant offspring a common question is: when should an individual invest in dormant offspring? In facultatively sexual species a common question is: why are some individuals more likely to invest in sex? For many species of *Daphnia*, there is additional complexity as investment in sexual reproduction and investment in dormancy are mechanistically linked. In this study, we explored the intraspecific variation that exists within and between populations of *Daphnia pulicaria* to understand why some populations and some individuals are more likely to invest in sexually-produced dormant offspring. We found support for our hypotheses that occasional bouts of sexual reproduction and/or production of dormant offspring for temporal dispersal may be beneficial. We explicitly linked two ecologically-important suites of traits with high intraspecific variation: a genotype's ability to grow on available resources and the genotype's likelihood of investing in sexually-produced dormant offspring. Our results demonstrate that the intraspecific variation in sex/dormancy investment

previously observed is due, in part, to a negative relationship with the expected active stage fitness of a genotype.

Figures and Tables

Table 2.1. Analysis of variance for investment in sexual reproduction by May-collected individuals from the six populations. Models were run using SAS Version 9.4, Proc GLM with persistence type (persisting vs. non-persisting population) and population of collection (nesting in type).

Source	d.f.	Mean Square	*F*-Value	*P*-Value
Sum investment in sexually-production and dormant offspring				$R^2 = 0.424$
Type	1, 4	0.932	5.89	0.072
Population (Type)	*4, 104*	*0.158*	*7.62*	*<0.0001*
Investment in male offspring				$R^2 = 0.387$
Type	1, 4	0.275	2.16	0.215
Population (Type)	*4, 104*	*0.158*	*7.62*	*<0.0001*
Investment in Ephippia				$R^2 = 0.551$
Type	1, 4	0.195	1.56	0.280
Population (Type)	*4, 104*	*0.125*	*24.33*	*<0.0001*

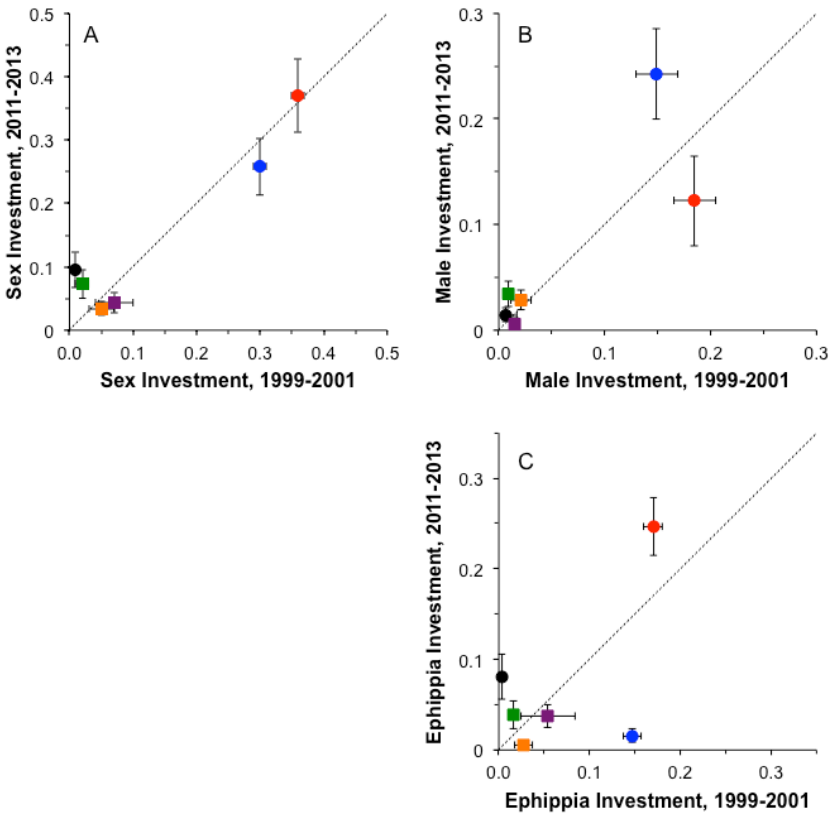

Figure 2.1. Decadal comparison of the proportion of sexual/dormant reproductive events from each population from the current assay (2011-2013) and proportion of field investment in sex/dormancy from Cáceres and Tessier's (2004a) (1999-2001). Each point is the May population mean for (A) sum investment in sexually-produced dormant offspring, (B) investment in male offspring, and (C) investment in ephippial offspring with one standard error representing the range in investment between genotypes in the populations. Non-persisting populations are circles (Baker = Black, Cloverdale = Red, Little Long = Blue) while persisting populations are squares (Bassett = Orange, Bristol = Purple, Warner = Green).

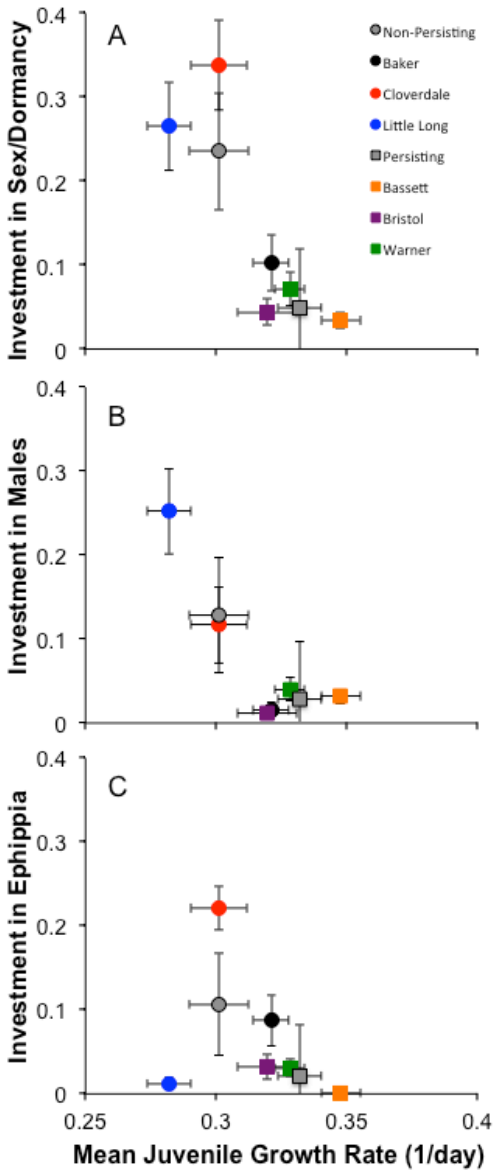

Figure 2.2. Relationship between population mean juvenile growth rate (a correlate of active stage fitness) and metrics of investment in sexually-produced dormant offspring (A) sum investment in sexually-produced dormant offspring, (B) investment in male offspring, and (C) investment in ephippia. Population types are denoted by shapes (persisting are squares; non-persisting are circles) and populations are color-coded. The mean for persisting and non-persisting populations are in grey. Each point is the mean with one standard error for intra-population variation.

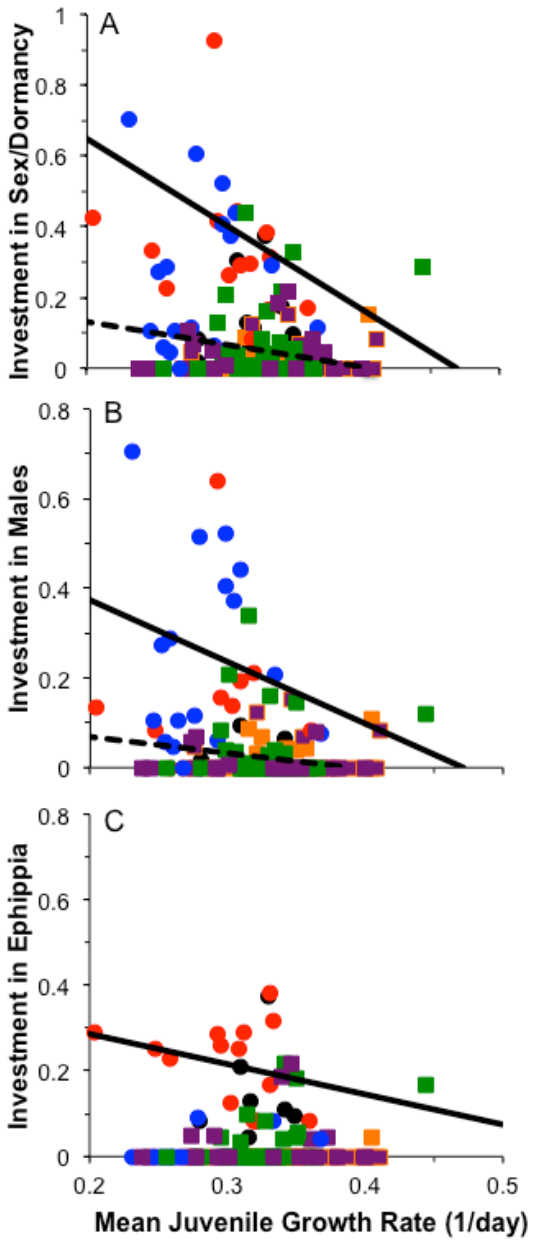

Figure 2.3. Relationship between mean juvenile growth rate and metrics of investment in sexually-produced dormant offspring at the individual level. Regression of genotype mean growth vs. (A) sum investment in sexually-produced dormant offspring, (B) investment in male offspring, and (C) investment in ephippia. Quantile regression lines shown for the 90th quantile (solid black line) and 50th quantile (LAD, dashed black line). Points represent the mean for each genotype collected and are coded by shape (persisting are squares; non-persisting are circles) and color (Baker = Black, Bassett = Orange, Bristol = Purple, Cloverdale = Red, Little Long = Blue, Warner = Green).

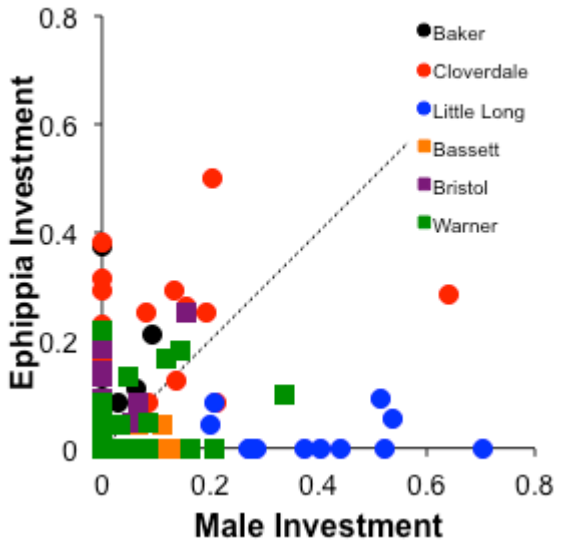

Figure 2.4. Genotype-level investment in males vs. investment in ephippia. Each point is the result of a genotype's mean investment in males (x-axis) and investment in ephippial clutches (y-axis). Points are color-coded by population of collection. Out of 172 assayed genotypes only 29 genotypes (16.9%) were generalists and invested in all three modes of reproduction (male/mixed clutches, ephippia, and asexual clutches).

Chapter 3: Sensitivity to changes in resource quality does not influence intraspecific competitive ability of *Daphnia pulicaria*

Abstract

Variation in ecologically important traits within consumer species may have implications for the outcome of intraspecific competition. One way that individuals may differ in competitive ability is through their ability to acquire and allocate resources. The power-efficiency trade-off predicts that there are individuals who experience fitness declines when resource quality declines while other individuals maintain equivalent fitness across a gradient of resource qualities. In this study, we use freshwater lakes, which have a predictable seasonal succession in the quality of algal resources from rich-quality in the spring to poor-quality in the summer, and genotypes of the zooplankton grazer *Daphnia pulicaria*, which differ in response to resource quality. We chose several powerful genotypes that are sensitive and efficient genotypes that are less sensitive to changes in laboratory-reared algal quality. Prior laboratory results show that powerful genotypes have greater growth rate on rich-quality algal resources and were therefore predicted to outperform efficient genotypes in the rich-quality resources of the spring. Instead, we found no difference in juvenile growth rate between high and low sensitivity genotypes when grown on field-collected resources, possibly due to low epilimnetic resource availability in both spring and summer assays. Additionally, we predicted that sensitive genotypes (individuals that grew rapidly on rich-quality laboratory diets) would dominate less sensitive and lower growing genotypes in competition trials on rich-quality resources. We found that high sensitivity genotypes did not have increased competitive ability on rich-quality resources. Despite the intraspecific variation in response to resource quality, sensitivity to resource quality does not appear to drive competitive dynamics in our short-term and low volume experiment. High population densities and overall resource limitation appear to constrain population densities and the effects of intraspecific competition appears to be equivalent whether or not a genotype is in intra-strain (against clones of the same genotype) or inter-strain (against other clones) competition.

Introduction

Intraspecific variation is common in ecologically-important traits (Bolnick et al. 2003, Bolnick et al. 2011, Violle et al. 2012) and often results in fitness differences of coexisting individuals (Weider 1985, De Meester et al. 1995, Tessier and Leibold 1997, Crawford et al. 2015). Even within species typically classified as a generalist, intraspecific variation means that there may be specialists who have higher fitness on certain resources and generalists who are better able to perform on a range of resource qualities or quantities (Tessier et al. 2000, Tessier and Woodruff 2002, Tinker et al. 2012, Terraube et al. 2014). One example of this intraspecific variation is the power-efficiency trade-off between "powerful" individuals who are better able to capitalize on abundant and/or rich-quality resources while other "efficient" individuals are better able to maintain growth on limited and/or poor-quality resources (Odum and Pinkerton 1955, Odum 1956, Smith 1976, Frederickson and Stephanopoulos 1981, Silvert 1982, Odum 1983, Watt 1986, Grover 1990, Raubenheimer and Simpson 1996, Tessier et al. 2000, Reznick et al. 2000). Powerful individuals are also considered "sensitive" to changes in resource quality as they suffer fitness declines when resource quality is poor (Hall et al. 2012). In contrast, efficient individuals are less sensitive to changes in resource quality and maintain equivalent fitness on both rich- and poor-quality resources.

In many temperate freshwater lakes, resource quality is limiting for herbivorous grazers as algae progress through a regular seasonal succession from rich-quality and readily digestible in the spring to poor-quality algae that are often toxic or digestion resistant in the summer (Sommer et al. 1986, Sterner and Schulz 1998, Sommer et al. 2012, Sarpe et al. 2014). Therefore, understanding how grazers respond to this change in resource quality is important for understanding population dynamics. High grazing pressure by *Daphnia* reduces the abundance of algal resources and *Daphnia* are in turn limited by reduced availability of resources, which can lead to cyclical dynamics in the laboratory (McCauley and Murdoch 1987, McCauley et al. 1988, Murdoch et al. 1998, McCauley et al. 1999). However, due to the change in resource quality between spring and summer (Sommer et al. 1986, Sommer et al. 2012), resource quality does not typically rebound in the field during the year and zooplankton experience variability in resource quality across seasons (De Stasio et al. 1995, Declerck et al. 2001). Field population densities of dominant grazers like *Daphnia pulicaria* are often correlated with this temporal heterogeneity in resource quality, with population density peaking in the rich quality resources of the spring and

declining in the summer coinciding with reduced resource availability, reduced resource quality, and other biotic pressures (Hutchinson 1967, Goulden and Hornig 1980, Tessier 1986, Kratz et al. 1987, Hu and Tessier 1995, Geedey et al. 1996, Cáceres 1998, Cáceres and Tessier 2004a).

Daphnia maintain both interspecific and intraspecific variation in response to resource quality (Gliwicz 1990, Tessier and Consolatti 1991, Vanni and Lampert 1992, Kirk et al. 1997, Hairston et al. 2001, Steiner 2005, Weider et al. 2005, Brzeziński and Von Elert 2007, Boon et al. 2007, Allen et al. 2012). Some *Daphnia* genotypes are powerful but sensitive to declines in resource quality while others are efficient and maintain similar growth on different qualities of algal resources (Hall et al. 2012, Chapter 1). As resource quality declines temporally from rich- to poor-quality, intraspecific variation between *Daphnia* individual's performances on rich- vs. poor-quality resources are predicted to influence a genotype's fitness. Therefore, a powerful/sensitive genotype may suffer a decline in fitness between the rich-quality resources of the spring and poorer-quality resources of the summer. In contrast, the fitness of an efficient genotype is predicted to be less effected by the changes in resource quality between spring and summer. While the efficient genotypes may not reach as high of fitness in the rich-quality resources of the spring, their fitness will not decline in the poor-quality resources of the summer, as is the case with the sensitive genotype.

Intraspecific variation in sensitivity to resource quality may influence the outcome of competition. Examples of interspecific competition are well documented in zooplankton (Bengtsson 1987, De Mott 1989, Rothhaupt 1990, Hu and Tessier 1995, Cáceres 1998, Steiner et al. 2007) and studies of intraspecific variation in algal-resource utilization by *Daphnia* have recently received more attention (Steiner et al. 2007, Brzeziński et al. 2010, Crawford et al. 2015, Chapter 1). Given the variation that exists within species and populations, there may also be intraspecific effects of variation in response to resource quality. One example of this variation is between fast growing powerful individuals who are sensitive to declines in resource quality and the slower-growing efficient individuals who are less affected by changes in resource quality. Therefore, the outcome of intraspecific competition may be influenced by differences in individuals' abilities to capture and allocate resources. Based on the predictions of the power-efficiency trade-off (Odum and Pinkerton 1955, Tessier et al. 2000, Hall et al. 2012), sensitive individuals are predicted to capitalize on rich-quality resources and dominate competition; however, these genotypes are sensitive to declines in resource quality and may lose in

competition to more efficient genotypes in poor-quality resources (as in Dudley 1996a, Dudley 1996b, Bolker and Pacala 1999, Tessier et al. 2000, Tessier and Woodruff 2002). These powerful but sensitive individuals may rapidly acquire rich-quality resources and allocate these nutrients to growth and reproduction and competitively exclude the efficient individuals. In contrast, the consistent growth of efficient individuals coupled with their low sensitivity to declines in resource quality means that they may be competitively dominant in poorer-quality resources as they can maintain growth and reproduction at a lower resource quality threshold (Hsu et al. 1977, Hsu et al. 1978, Armstrong and McGehee 1980).

In this study, we build on prior research focused on the importance of intraspecific variation in algal-resource utilization to predict the outcome of intraspecific competition and performance on field-collected resources. We chose six genotypes (previously assayed in Chapter 1) that had either high or low sensitivity to resource quality as measured by their growth on rich- and poor-quality laboratory-reared algal diets. To determine performance on natural algal resource assemblages, we used a juvenile growth rate assay on field-collected resources. Juvenile growth rate is a common proxy for fitness in *Daphnia* (Tessier and Goulden 1987, Tessier et al. 2000, Hairston et al. 2001, Hall et al. 2012, Robinson and Beckerman 2013, Crawford et al. 2015). Based on the predictions of the power-efficiency trade-off (Odum and Pinkerton 1955, Tessier et al. 2000, Hall et al. 2012) and the seasonal succession of resource quality (Sommer et al. 1986, Sommer et al. 2012), we predicted that high sensitivity genotypes would grow comparatively better in spring-collected resources compared to summer-collected resources. As sensitive genotypes grow well on rich-quality resources but their fitness declines on poor-quality resources (Tessier et al. 2000, Hall et al. 2012, Chapter 1), we predicted that sensitive genotypes would dominate competition on rich-quality diets and be the inferior competitor on poor-quality diets. In this simple consumer-resource system, short-term population dynamics may be directly driven by the ability of individuals to acquire resources and allocate them to growth and reproduction. Powerful-but-sensitive individuals are predicted to capitalize on rich-quality resources and may therefore dominate competition in rich-quality treatments. We predicted that these competition outcomes would be due to differences in population growth rate (r) and birth rate (b) in which high sensitivity genotypes would have high r and b only in rich-quality diets while low sensitivity genotypes would maintain similar r and b in both diet treatments.

Methods

Experiment Preparation and Clone Selection

We selected clones from those used in a previous study that assayed the juvenile growth rate (JGR) of *Daphnia pulicaria* individuals on two qualities of green algal diets: rich-quality *Ankistrodesmus falcatus* and the poor-quality digestion resistant *Oocystis* B (Chapter 1). The JGR assay is commonly used as a proxy of fitness for *Daphnia* (Tessier and Goulden 1987, Lampert and Trubetskova 1996, Desmarais and Tessier 1999, Tessier et al. 2000, Hairston et al. 2001, Tessier and Woodruff 2002, Allen et al. 2010, Crawford et al. 2015). Here, we calculated each genotype's sensitivity to resource quality by finding the slope of the line between JGR on rich- vs. poor-quality diets against the growth on each diet by a standard *Daphnia* grazer (the *D. pulex-pulicaria* hybrid as in Hall et al. 2012; see also Tessier et al. 2000, Tessier and Woodruff 2002). Individuals with steeper slopes had greater differences in JGR between the rich- and poor-quality diets; therefore, those individuals were deemed more sensitive to changes in resource quality (as in Hall et al. 2012). From the previously assayed genotypes, we chose three genotypes each for high vs. low sensitivities (Fig. 3.1A; Table 3.1). For this study, all genotypes were specifically chosen with equivalent mean juvenile growth rate so that we focus only on the effect of sensitivity to resource quality (Fig. 3.1B; Table 3.1). The difference in sensitivity to resource quality is driven by a higher mean JGR on the rich-quality diet by the high sensitivity individuals (Fig. 3.1C; Table 3.1). Intra-genotype variation in the high sensitivity genotypes on poor-quality resources resulted in both high and low sensitivity genotypes with equivalent growth rates on the poor-quality diet (Fig. 3.1D; Table 3.1). As the three genotypes of each sensitivity do not differ (Table 3.1, no effect of Genotype(Sensitivity)), we use the mean of the three-genotype mean response in all subsequent analyses.

Field Assay of Juvenile Growth Rate

To test our prediction that more sensitive genotypes will have higher juvenile growth rate when grown on the spring-collected resources compared to less sensitive genotypes, we used a modification of the juvenile growth rate (JGR) analysis (Tessier and Goulden 1987, Tessier et al. 2000, Allen et al. 2010, Crawford et al. 2015, Chapter 1). Prior to the start of each experiment, reproductive individuals were transferred to holding containers devoid of laboratory-raised algal resources so that neonates would not feed between birth and their experimental treatment. For

the natural algal assemblage, we collected epilimnetic water from Sportsman's Lake (Vermilion County, IL) using an integrated tube sampler one meter past the chlorophyll-*a* maximum (5m in May and 4m in July, Fig. E.1). Chlorophyll-*a* was extracted according to Welschmeyer (1994): 100mLs of lake water from each depth were filtered over a GF-F Filter, filters were eluted in 10mLs of 100% EtOH overnight at 4°C, then assessed in a Turner Designs Trilogy Flurometer (Turner Designs, Sunnyvale, CA, USA). Total spring vs. summer chlorophyll-*a* abundance in this epilimnetic sample was calculated using the standard trapezoid rule and difference in spring vs. summer abundance was assessed via a T-Test.

Neonates born within 18 hours of the start of the experiment were split into two treatments. Some individuals ($N \geq 10$) were immediately dried in a 60°C oven overnight for initial mass. The remaining individuals ($N \geq 21$) were set up at a density of three per 200mL water. This design was replicated three times in each season. Beakers were stored in an environmental chamber at standard laboratory conditions (20°C; 14[light]:10[dark] photoperiod). Fresh lake water was collected daily, screened with 76μm mesh to remove zooplankton, and individuals were transferred into newly collected and filtered water every afternoon for the duration of the experiment. At the end of the experiment, individuals were dried overnight and masses were taken on a Mettler Toledo UMX2 microbalance. Juvenile growth rate was then calculated using Tessier and Goulden (1987)'s formula:

$$\text{Juvenile Growth Rate} = \frac{\ln(\text{mass on day five}) - \ln(\text{mass on day zero})}{\text{days of growth}}$$

We assessed the ability of individuals of both sensitivities to grow on spring- and summer-collected resources using an ANOVA: fixed effects sensitivity (high or low) and unique genotype (nested in sensitivity). To test our hypotheses that high sensitivity genotypes would grow well in spring- and poorly on summer-collected resources, we used an ANOVA (SAS, Version 9.4, Proc GLM) with season of resource collection and sensitivity to resource quality as fixed effects and tested for a sensitivity-by-season interaction. A sensitivity-by-season interaction indicates that there is a trade-off between the two sensitivities' performance on spring- vs. summer-collected resources. We also used a regression (SAS, Version 9.4, Proc REG) to determine if sensitivity to resource quality in the lab was predictive of growth rate on field-collected resources.

Laboratory Assay of Competitive Ability

To test whether sensitivity to resource quality influences short-term competitive ability on the two lab-reared algal diets, we used a laboratory-based competition assay in 800mL experimental containers. Fifty-four containers (two sensitivities [high vs. low] x three genotypes per sensitivity x two diet qualities [rich vs. poor] x three replicates) were stocked with an initial density of three individuals per sensitivity. We also set up monocultures at a density of 6 individuals. Each day we inoculated beakers with a limiting density (0.5mg/L) of either the rich-quality *Ankistrodesmus falcatus* or the digestion resistant poor-quality *Oocystis* B. *Oocystis* was conditioned according to De Mott et al. (2010) and Hall et al. (2012) to form a gelatinous sheath that renders the algae digestion resistant. On days six, nine, 13, and 16 containers were thoroughly agitated and 400mLs water and algae were removed to further limit resource availability. On each of those days we tracked population density by counting all *Daphnia* individuals that were removed with that subsample before returning those individuals to the experimental container with 400mLs of freshly filtered water. On day 21, *Daphnia* were sieved out of the containers and preserved in 100% EtOH for final population density counts and molecular analysis. From the preserved samples, we exhaustively counted final density. To distinguish genotypes in the competition assay, 20 individuals (juvenile and adult) were haphazardly selected from the preserved samples. We used the microsatellite marker technique previously described in Chapter 1 to assess genotypes at six loci for the *Daphnia pulex* complex (Dp 27, 75, 102, 196, 433, 461; full methods in Appendix F). From the genotyping results, we calculated the proportions of high and low sensitivity individuals in the final density.

To test the hypothesis that high sensitivity genotypes would be at a greater density (indicating competitive superiority) on the rich-quality diet, we used an ANOVA (SAS, Version 9.4, Proc GLM) with diet (rich-quality *Ankistrodesmus* vs. poor-quality *Oocystis*) and sensitivity (high vs. low) as fixed effects and tested for a sensitivity-by-diet interaction, which would indicate a trade-off in competitive ability on the two diets. We did not use genotype in the subsequent analyses due to the lack of difference between genotypes within each sensitivity (Table 3.1). We ran the same analysis for the monoculture density. To test for intra-strain competition influencing density and to compare the monoculture density to the competition density, we used and ANOVA (SAS, Version 9.4, Proc GLM) with diet, treatment (monoculture vs. competition), and a diet*treatment interaction as fixed factors.

We also calculated population growth rate (r) and birth rate (b). Population growth rate was calculated as:

$$r = \frac{\ln(\text{Final Density}) - \ln(\text{Initial Density})}{\text{Time}}$$

We noticed that there was an inflection point at day 13 when density for the rich-quality diet plateaued but the poor-quality diet treatment kept rising (Fig. F.1); we therefore used a repeated measures (rm)ANOVA (SAS, Version 9.4, Proc GLM) to test for the effect of this plateau in density increase. We assessed the effect of time (r 0-13 days and r 13-21 days) as well as sensitivity to resource quality, diet, and their interaction. A significant time-by-diet interaction suggests that r is different for the two diet qualities before and after day 13. Egg ratios were used to estimate population birth rate (b) on day 21 of the experiment and were calculated according to Edmondson (1960), Paloheimo (1974), and Cáceres (1998):

$$b = \frac{\ln((E/N) + 1)}{D}$$

In which birth rate (b) equals the number of eggs (E) being carried by the number of adult females in the population (N) at the temperature dependent development time (D). Temperature dependent development time was calculated from Gulbrandsen and Johansen (1990). As we could only estimate egg ratios from the preserved samples, we can only calculate b for day 21 and therefore do not have an equivalent time-series analysis for birth rate. We used an ANOVA (SAS, Version 9.4, Proc GLM) with sensitivity to resource quality, diet, and their interaction as fixed effects.

Results

Field Assay of Juvenile Growth Rate

The quantity of epilimnetic algal resources collected from Sportsman's Lake in both May and July was very low and chlorophyll-*a* from integrated tube samples was lower in May (1.61 ±0.06µg/L) than July (2.33 ±0.02µg/L) (T-Test, t = -11.680, $p < 0.001$). Despite this difference in total chlorophyll-*a*, the abundance is near starvation levels (Sterner and Schulz 1998). Supporting this, a clone used as a bioassay (the Geedey *Daphnia pulex-pulicaria* hybrid as in Tessier and Woodruff 2002, Hall et al. 2012) did not differ in its JGR between spring-collected resources (May Mean JGR = 0.154 ±0.041) and summer-collected resources (July Mean JGR =

0.113 ±0.017) (Fig. 3.1E-F; T-Test, t = 0.918, $p = 0.495$). The Geedey clone also had much lower juvenile growth rate on field-collected resources (Fig. 3.1E-F) than it did on laboratory-raised resources (Fig. 3.1C-F).

Contrary to our prediction that high and low sensitivity genotypes would differ in their performance on resources collected from the field, we found no difference in the response to the spring- vs. summer-collected resources. Juvenile growth rate (JGR) of both sensitivities were equivalent in both spring- and summer-collected resources (Fig. 3.1E-F; Table 3.2). We did not support a predicted sensitivity-by-season interaction (Fig. 3.2A; ANOVA, $F_{1, 8} = 0.02$, $P = 0.882$) indicating that high sensitivity genotypes do not have higher mean JGR in the spring and lower mean JGR in the summer, compared to low sensitivity genotypes. In fact both high and low sensitivity genotypes had equivalent overall JGR (Fig. 3.2A; ANOVA, $F_{1, 8} = 0.58$, $P = 0.469$). As with the growth rate of the standard bioassay clone presented above, we found that spring- and summer-collected resources yielded equivalent JGR (ANOVA, $F_{1, 8} = 4.61$, $P = 0.064$).

We also found no support for our prediction that clones with higher sensitivity would perform better in field-collected resources in the spring and worse in resources collected in the summer (Fig. 3.2B-C). Our regression analysis demonstrates that sensitivity to resource quality does not influence a genotype's mean JGR on May-collected resources (Fig. 3.2B; Regression, t = 0.84, $P = 0.448$) or July-collected resources (Fig. 3.2C; Regression, t = 0.32, $P = 0.768$). In sum, laboratory sensitivity to resource quality did not predict growth on field-collected resources.

Laboratory Assay of Competitive Ability

In our competition assay, we found that the sensitivity to resource quality of a genotype did not influence the outcome of competition (Fig. 3.3). Due to higher juvenile growth rate on rich-quality resources (Fig. 3.1C; Table 3.1), we predicted that high sensitivity genotypes would outcompete low sensitivity genotypes in rich-quality diets. Density in monocultures revealed no sensitivity-by-diet trade-off (Fig. 3.3A; ANOVA, $F_{1, 8} = 0.49$, $P = 0.503$), no difference in density based on sensitivity to resource quality (Fig. 3.3A; ANOVA, $F_{1, 8} = 0.02$, $P = 0.902$), and no difference in density in both rich- and poor-quality diets (Fig. 3.3A; ANOVA, $F_{1, 8} = 2.17$, $P = 0.179$). We did not support the predicted trade-off in density in the different diets in competition

via the sensitivity-by-diet interaction (Fig. 3.3B; ANOVA, $F_{1, 22} = 1.05$, $P = 0.314$). Instead, we found that final density in competition was equivalent for both high and low sensitivity genotypes (Fig. 3.3B; ANOVA, $F_{1, 22} = 1.25$, $P = 0.271$) and that overall density did not differ between diet treatments (rich- vs. poor-quality diet) (Fig. 3.3B; ANOVA, $F_{1, 22} = 2.91$, $P = 0.098$). When comparing the total monoculture densities to the competition densities, we found that there was no effect of treatment (density reached in monoculture was equivalent to overall density reached in competition; ANOVA, $F_{1, 26} = 1.52$, $P = 0.229$). We also found no diet-by-treatment interaction (ANOVA, $F_{1, 26} = 0.04$, $P = 0.837$); therefore, genotypes were not influenced by whether competition was inter-strain (high sensitivity vs. low sensitivity) or intra-strain (competition due to high population density in the monoculture). In the analysis between monoculture and competition population densities, we did find that mean density on day 21 was higher in the poor-quality diet (ANOVA, $F_{1,26} = 12.58$, $P = 0.002$); but see below for predictions of future population densities based on birth rates calculated on day 21.

Although overall density did not differ between treatments (rich- vs. poor-quality algal diets) in the competition assay, there were several interesting trends with population growth rate (r) in the monocultures (Fig. 3.4). Population growth rate in the rich-quality diet initially rises and then plateaus at day 13 of the experiment (Fig. F.1). We broke our analysis of r into two time points based on the plateau (r for days 0-13 vs. r for days 13-21) and found that r differed in the two diet treatments during the last eight days of the experiment (Table 3.3). In the monocultures, although overall population density did not differ in the between-subjects effects for sensitivity or diet quality, r declined in the rich-quality diet after day 13 but increased in the poor-quality diet (Fig. 3.4A; Table 3.3). Monoculture population growth rate of high and low sensitivity genotypes responded in the same way to diet quality (Fig. 3.4A; Table 3.3).

While population growth rate trends at the end of the competition experiment suggest an increase in density in poor-quality diets, the birth rate (b) at day 21 indicates that a population density plateau or crash is imminent (Fig. 3.4B). Individuals grown in the poor-quality diet had higher birth rates at day 21 compared to those grown in the rich-quality diet, as measured by egg ratio counts (Fig. 3.4B; ANOVA, $F_{1, 8} = 8.12$, $P = 0.022$). These results were equivalent for both high and low sensitivity individuals (Fig. 3.4B; ANOVA, $F_{1, 8} = 0.35$, $P = 0.569$). No diet-by-sensitivity interaction in birth rate indicates that high and low sensitivity individuals responded equivalently to the two diet qualities (Fig. 3.4B; ANOVA, $F_{1, 8} = 0.12$, $P = 0.733$). But note that

the overall low birth rate in both diet treatments suggest that the density reached on day 21 will soon plateau and population density may begin to decline.

Discussion

Despite prior results that genotypes differed in performance on rich-quality laboratory-reared algal resources, we did not support our predictions that a genotype's sensitivity to resource quality would influence juvenile growth rate (JGR) on a natural algal assemblages or influence competitive ability. Specifically, our field growth assay failed to support our prediction that high sensitivity genotypes would have higher growth on a spring-collected compared to a summer-collected algal assemblage. In the competition assay, we found no support for our prediction that high sensitivity genotypes would outcompete low sensitivity genotypes in a rich-quality diet treatment. Density was similar at the end of the 21-day competition experiment in both rich- and poor-quality diets, despite different population growth rate dynamics in the two diet qualities. Although population growth rate was higher at the end of the experiment in the poor-quality diet, the very low birth rate suggests that a population density crash, or at least plateau, is imminent. Given that the final densities of both high and low sensitivity genotypes were equivalent, high sensitivity genotypes do not have a short-term competitive advantage on rich-quality resources. Furthermore, it appears that the effects of competition are equivalent whether competing inter-strain (high vs. low sensitivity genotypes) or intra-strain (against clonal sisters).

We based our prediction that sensitivity to resource quality would influence juvenile growth rate in different seasons on previous results that algal resource quality declines from spring to summer in freshwater populations (Sommer et al. 1986, Sommer et al. 2012). Individuals who were able to maximize growth on laboratory-reared algae were predicted to also capitalize on spring resources but have comparatively lower juvenile growth on summer resources. The most likely explanation for our result is the very low abundance of epilimnetic algal resources. The epilimnetic chlorophyll-*a* abundance in Sportsman's Lake in May and July were close to the starvation threshold of 1.8μg/L and well below the maximum growth rate threshold of 6-15μg/L calculated by Sterner and Schulz (1998). As our clones had equivalent performance on poor-quality resources in the laboratory and prior assays were run on non-

limiting quantity, it is possible that our genotypes were equally affected by the low overall abundance of resources. Our standard bioassay clone also maintained equivalently low overall growth on both spring- and summer-collected resources further suggesting that starvation levels of algal resources in both spring and summer collections limited growth in this experiment. Our results align with prior research by Sarpe et al. (2014) who found that the limited quantity of rich-quality resources from lakes negatively influenced *Daphnia galeata* survival, growth, and reproduction. Furthermore, resources abundance deemed "limiting" by Sarpe et al. (2014) still had higher chlorophyll-*a* concentrations than our lake; indicating that our source for algal resources was severely limited in resource quantity. Therefore, even if resource quality is rich in the spring, low quantity of resources can still limit the growth rate of juvenile *Daphnia.* We intentionally sampled through the chlorophyll-*a* maxima as *Daphnia* vertically migrate to feed in bands of dense resources; however, it is possible that *Daphnia* native to this lake feed almost exclusively in dense bands of algal resources to avoid the very low abundance of algae in much of the epilimnion (Geller 1986, Gliwicz 1986, Johnsen and Jakobsen 1987, Stitch and Lampert 1984, Leibold 1990).

Given that our competition assay was a simple consumer-resource system, we predicted that short-term population dynamics would be directly driven by resource limitation and the ability of individuals to capture resources via exploitative competition. Therefore, powerful individuals were predicted to be competitively dominant on rich-quality resources as their growth rate is higher on rich-quality resources compared to efficient individuals. In contrast, efficient individuals, who are less sensitive to poor quality resources, were predicted to perform comparatively better in a poor-quality resource treatment. In the laboratory-based competition assay, we predicted that overall density would be higher in the rich-quality treatment and that sensitive individuals would have a greater relative density in rich- and lower relative density in poor-quality treatments. We found that density was equivalent for both sensitivities in both diet treatments. Our predictions were based on prior work by Weider et al. (2005) who showed that the nutritional quality of algal resources could be an important driver in the outcome of competition between *Daphnia* clones. Three factors may influence the results that we observed in the competition assay: (1) a change in feeding rate when resources became limiting, (2) a change in the pace of life in rich- and poor-quality resources, and/or (3) maternal provisioning better prepared offspring for survival in the poor-quality treatment. A review by Vasseur et al.

(2014) found that zooplankton competitor species (at the interspecific level) often responded similarly to a single environmental stimulus, such as resource limitation. Therefore, it is possible that the trends observed in this study were equivalent at the intraspecific level; both high and low sensitivity individuals may be responding equivalently to the stress of resource limitation.

A change in the feeding behavior of *Daphnia* individuals may explain why there is a plateau in the population growth rate in the rich-quality competition treatment. Prior results by Weber (2001) and Lurling et al. (2003) show that *Daphnia* reduce their feeding rate when population density is high. Due to the rapid rise in population density in the rich-quality competition diet, it is possible that the plateau was a result of a shift in resource acquisition by all individuals in the rich-quality diet. As filtering rate decreases in high densities and individuals have fewer resources to allocate for reproduction, birth rate and population growth rate would slow. Fitzsimmons and Innes (2006) demonstrated that *Daphnia* reduce their production of clonal daughters at higher population densities. Fitzsimmons and Innes (2006) and Koch et al. (2009) found that production of ephippia (haploid eggs awaiting fertilization) and adult male production also increases in high-density *Daphnia* cultures. While we did not track male production, we did notice an increase in ephippia production around day 13, when the population density in the rich-quality treatment began to plateau. This change in feeding behavior may account for the reduction in population growth rate in the rich-quality treatment around day 13 as population density reached carrying capacity. The later plateau in the poor-quality treatment may be due to the longer period of time before these populations reached equivalently high density.

Daphnia also reduce their feeding rate when resource availability declines (Penczykowski et al. 2014). Given the rapid rise in population density especially in the rich-quality treatment, *Daphnia* rapidly became resource limited. Competition experiments require resource limitation to determine competitive dominance, but this resource limitation may have had the additional effect of altering the feeding rate of individuals in competition. As a result of the reduced feeding rate, less energy was available for reproduction and may explain the reduction in population growth rate after day 13 in the rich-quality treatment. In the poor-quality treatment, individuals reached the same densities later on in the experiment, which may explain why the plateau had not yet occurred. Given the low birth rate on day 21, it appears as though that limitation based on resource quality would soon cause the population density in the poor-quality treatment to plateau or even crash. Our study also included several age classes that were

all exerting grazing pressure on the available algal resources in the competition containers. Results by McCauley et al. (2008) indicate that neonates, juveniles, and adults may exert different grazing pressures on resources. As juveniles develop at different rates and adults eventually die, grazing pressure is not always constant on algal populations (Ananthasubramaniam et al. 2011) and these different age classes can allow algal population density to recover (Nelson et al. 2007). As we had multiple age classes simultaneously grazing, variation in consumers would maintain strong grazing pressure on the limited algal resources such that the *Daphnia* were constantly suppressing their resources once they reached a great enough density.

A change in the pace of life of individuals may also help explain why we observed the counterintuitive pattern that population growth rate remained high in the poor-quality resource treatment and that both diet treatments reached equivalent densities at day 21. After the initial rise in population density, r slowed in the rich-quality diet and increased in the poor-quality diet. Why would r be higher in poor-quality environments? One explanation is offered by the "live-fast, die-young hypothesis" in which offspring production will be maximized when adult mortality is higher (Stearns and Carandall 1981). In poor-quality environments, Roff (1982) and Brough and Dixon (1989) both demonstrated that offspring production often supersedes adult maintenance in importance of energy allocation. However increased reproduction often comes at a cost; Snell and King (1977) and Bell (1984) both showed that rotifers that produced more offspring had earlier mortality. While there is variation in the "live-fast, die-young" trend, the relationship between adult maintenance and reproductive investment is usually negative when individuals are under resource stress (Partridge and Harvey 1985, Reznick 1985). When in stable and rich-quality environments, Goulden and Hornig (1980) and Boyce and Perrins (1987) both found that an intermediate (low-risk, low gain) clutch size has the highest geometric mean fitness. Therefore, consistent intermediate levels of reproduction have the highest mean fitness over a longer period, although this is dependent on the individual surviving to subsequently reproduce. Therefore, individuals in poor quality environments are more likely to invest in numerous offspring although they are more likely to die earlier.

Finally, offspring of some species may be better prepared for surviving in a poor-quality environment through maternal provisioning. This may explain why individuals in the poor-quality treatment were able to survive and subsequently reproduce. Garbutt and Little (2014,

2017) found that *Daphnia magna* offspring from food-stressed mothers are generally larger and feed slower than offspring from well-provisioned mothers. Larger and better-provisioned offspring are more likely to outperform offspring from mothers who were not resource-stressed (Smith and Fretwell 1974, Wilson and Lessells 1984, Parker and Begon 1986, Godfray 1987, Lloyd 1987). These offspring are also more likely to be starvation resistant than their parents (Tessier et al. 1983). As a result of maternal provisioning, influencing the starvation resistance of offspring, it is possible that individuals from our poor-quality treatment were better provisioned and fed slower than their counterparts in the rich-quality treatment. Therefore, provisioning provides offspring with an increased chance of survival; this may have allowed the population density in the poor-quality treatment to reach equivalent densities as the rich-quality treatment, although with a population decline predicted by the low birth rate, densities may not remain equivalent in the long term.

In our experimental design, we tested the influence of sensitivity on the outcome of short-term competition through rapid exploitation of resources and population density increase; however, an individual's response to poor environmental or resource conditions may matter more to the outcome of longer-term competition. In long-term intraspecific competition, the individuals that perform the best on poor-quality resources may outperform the individuals who do comparatively better in rich-quality resources. Schaum and Collins (2014) found that fast growing individuals (akin to our powerful-but-sensitive genotypes) are poor long-term competitors and are easily stressed by reduction of resource quality. Additionally, individuals with a more efficient growth rate were better competitors in the long term as they aren't overrunning nutrients and are less stressed by changes in resource quality (Schaum and Collins 2014). Mazer and Schick (1991a, 1991b) also support the trend that more efficient plant genotypes generally prevail in competition with faster growing genotypes. McGill et al. (2006) and McGill (2012) demonstrated that populations that are more tolerant to environmental variability and poor conditions are the ones that win in competition. Therefore efficient genotypes, those that are not sensitive to changes in resource quality, may dominate competition in the long-term as these individuals are not sensitive to changes in resource quality and are less likely to overrun available resources.

In this simple consumer-resource system, we predicted that short-term competitive dynamics may be driven by individuals' sensitivity to resource quality; but, in longer-term

studies, other outcomes could arise. Long experiments in the *Daphnia*-algae system has revealed that population dynamics may begin to cycle with initial suppression of algae followed by overexploitation of resources leading to subsequent *Daphnia* crash (McCauley and Murdoch 1987, McCauley et al. 1988, Murdoch et al. 1998, McCauley et al. 1999, Nelson et al. 2007). Prior studies have shown that this fluctuation in resource through time may help maintain multiple competitors (Koch 1974, Armstrong and McGehee 1976, Hsu et al. 1977, Hsu et al. 1978, Armstrong and McGehee 1980). Therefore, future studies of competition could also study both the short-term and long-term population dynamics of intraspecific competition by repeatedly genotyping experimental populations and tracking whether a genotype eventually rises to dominance or whether both sensitivities are maintained.

We demonstrated that the outcome of short-term competition was not driven by intraspecific variation in genotypes' sensitivity to resource quality. Although there is high genotypic and phenotypic variation in *Daphnia* (Geedey et al. 1996, Allen and Lynch 2008, Allen et al. 2010) and some of this variation is in an individual's sensitivity to resource quality (Chapter 1), we do not support our prediction that this individual-level variation influences differences in competitive ability. Our results demonstrate the importance of resource limitation that was rapidly reached in a rich-quality environment and how this limitation reduced population growth rate. Counter-intuitive trends in poor-quality environments may have been due to increased offspring production (explained by the "live-fast, die-young" hypothesis) and offspring survival due to maternal provisioning. Therefore, factors other than response to resource quality likely influence the outcome of intraspecific competition.

Tables and Figures

Table 3.1. Effect of sensitivity (high vs. low) and unique genotype (nested in sensitivity) on each metric of sensitivity to resource quality and juvenile growth rate (JGR).

Effect	d.f.	Mean Square	F-Value	*P*	
Response: Sensitivity to Resource Quality					$R^2 = 0.429$
Sensitivity	*1*	*7.634*	*39.72*	*0.003*	
Genotype (Sensitivity)	4	0.192	0.32	0.858	
Residual	18	0.592			
Response: Mean Juvenile Growth Rate					$R^2 = 0.077$
Sensitivity	1	0.001	0.42	0.553	
Genotype (Sensitivity)	4	0.012	0.35	0.839	
Residual	18	0.009			
Response: Juvenile Growth Rate on Rich-Quality Diet					$R^2 = 0.280$
Sensitivity	*1*	*0.042*	*8.01*	*0.047*	
Genotype (Sensitivity)	4	0.005	0.64	0.638	
Residual	18	0.008			
Response: Juvenile Growth Rate on Poor-Quality Diet					$R^2 = 0.152$
Sensitivity	1	0.018	4.06	0.114	
Genotype (Sensitivity)	4	0.004	0.40	0.805	
Residual	18	0.011			

Table 3.2. Effect of sensitivity (high vs. low) and unique genotype (nested in sensitivity) on Juvenile Growth Rate (JGR) of clones grown on resources collected in the field in spring (May) and summer (July).

Effect	d.f.	Mean Square	F-Value	*P*	
Response: JGR in May					$R^2 = 0.323$
Sensitivity	1	0.002	0.22	0.663	
Genotype (Sensitivity)	4	0.036	1.36	0.306	
Residual	12	0.007			
Response: Mean Juvenile Growth Rate					$R^2 = 0.077$
Sensitivity	1	0.004	0.36	0.582	
Genotype (Sensitivity)	4	0.012	0.95	0.471	
Residual	12	0.013			

Table 3.3. Repeated measures (rm)ANOVA for the effect of time on population growth rate (r). The significant Time-by-Diet effect supports the reduction in r observed in the rich-quality diet and demonstrates that r changed in the monoculture between the first 13 days and the last eight.

Source	d.f.	Mean Square	F-Value	*P*
Between-Subjects Effects				
Sensitivity	1	0.00007	0.08	0.783
Diet	1	0.00386	4.33	0.071
Sensitivity * Diet	1	0.00079	0.88	0.375
Residual	8	0.00713		
Within-Subjects Effects				
Time	1	0.00060	0.43	0.528
Time * Sensitivity	1	0.00001	0.01	0.928
*Time * Diet*	*1*	*0.01844*	*13.32*	*0.007*
Time * Sensitivity * Diet	1	0.00003	0.02	0.887
Residual	8	0.00138		

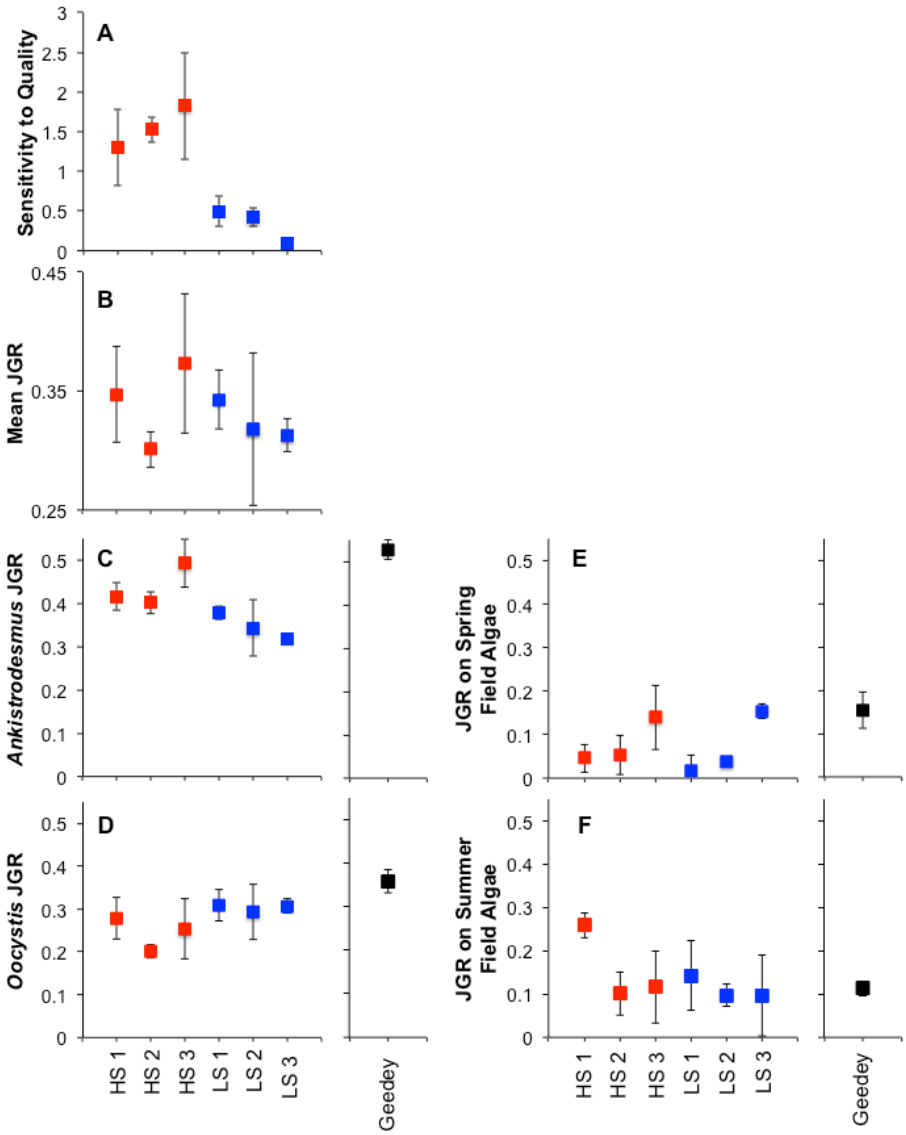

Figure 3.1. Individual metrics of sensitivity to resource quality and juvenile growth rate (JGR) for the three high sensitivity (red) and three low sensitivity (blue) clones from prior assay (Chapter 1). Genotypes were specifically selected for differences in sensitivity to resource quality (A) while maintaining equivalent mean JGR (B). The difference in mean sensitivity was driven performance on the rich-quality *Ankistrodesmus* diet (C), not on poor-quality *Oocystis* B diet (D). Each point is the genotype mean with 1 standard error. Individual metrics of JGR for the clones, including the Geedey bioassay clone, grown on spring collected (E) and summer collected (F) resources.

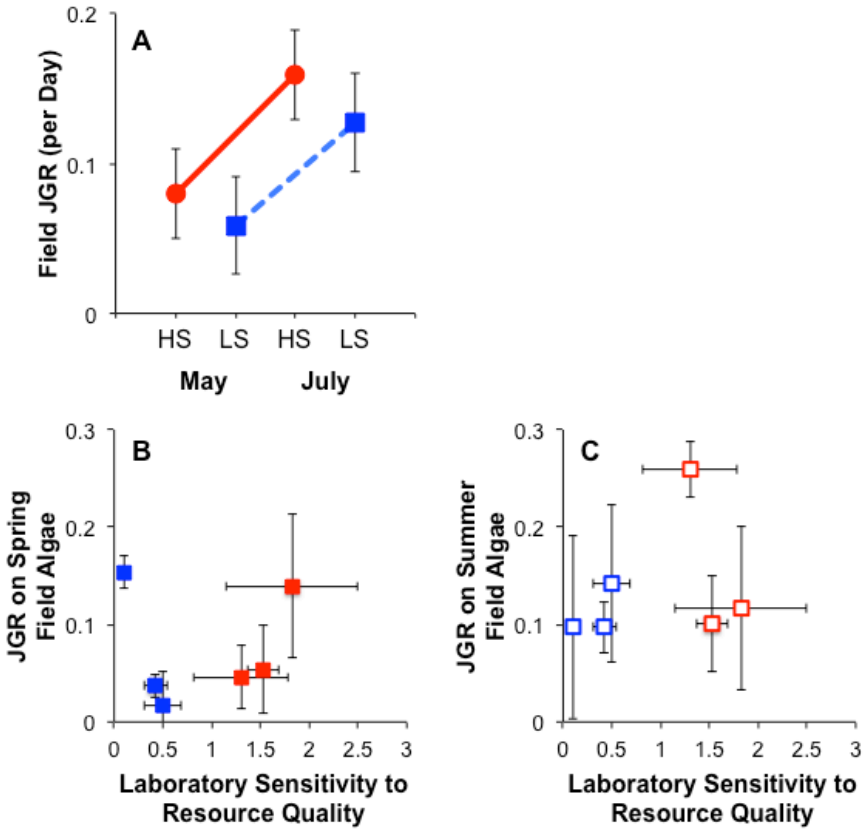

Figure 3.2. Response to field resource quality. High sensitivity genotypes are red while low sensitivity genotypes are blue. (A) Mean juvenile growth rate (JGR) of high sensitivity (circles, solid line) and low sensitivity (squares, dashed line) individuals grown on field-collected resources. JGR for May-collected resources on the left; JGR for July-collected resources on the right. Points are the mean JGR per strategy with 1 standard error. No relationship between laboratory sensitivity to resource quality and JGR on spring-collected resources (B) or summer-collected resources (C).

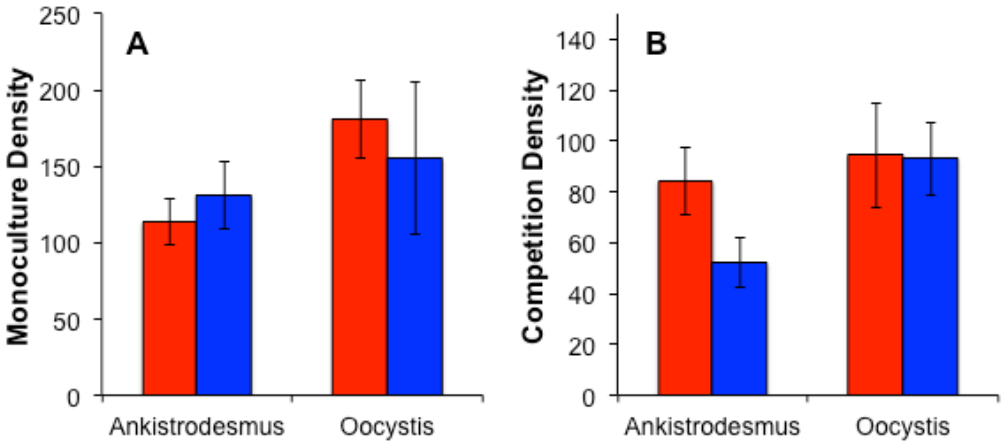

Figure 3.3. (A) Population densities of each sensitivity in the monoculture diets. (B) Proportions of genotypes of each strategy the competition diet (*Ankistrodesmus* = rich-quality; *Oocystis* = poor-quality digestion resistant) on day 21. Mean density for High Sensitivity genotypes (red) and Low Sensitivity genotypes (blue) with 1 standard error.

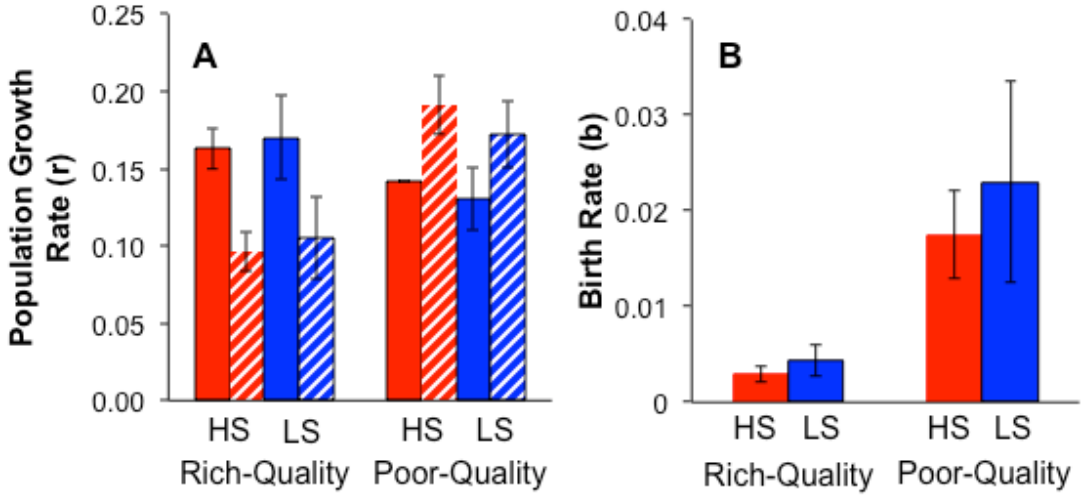

Figure 3.4. (A) Mean population growth rate (r) for each strategy in rich- vs. poor-quality resources in the monocultures. There was an inflection point in the data at day 13 of the experiment as densities in rich-quality (*Ankistrodesmus*) diet stopped rising, therefore the figure is broken down into r for days 0-13 (solid bars) and r for days 13-21 (dashed bars). (B) Population mean birth rate (b) on each diet in the competition treatments on the final day of the experiment (day 21). Note the very low birth rate in both treatments on day 21. Each bar is the mean for each sensitivity with 1 standard error.

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Appendix A: Additional molecular methods.

We assessed the genetic variation in the field collected iso-female lines through microsatellite analysis (a modification of Cristescu et al. 2006, Allen et al. 2010, Holmes et al. 2016). Tissue digestion and DNA extraction was completed using Qiagen DNeasy Blood & Tissue Kit. Whole *Daphnia* individuals were digested in 20µl of proteinase K and 180µl of ATL (tissue lysis) buffer and then incubated at 37°C for a minimum of 14 hours and a maximum of 20 hours. DNA was extracted by adding 200µl of EtOH and 200µl AL (lysis) solution to a Qiagen spin column and then centrifuged. Filtrate was washed twice with Qiagen solutions AW1 and AW2, both with centrifugation immediately after wash. DNA was removed from the filter using 30µl of AE (elution) buffer and washed into a clean tube. Six microsatellite loci for the *Daphnia pulex* (Dp) complex (Dp 27, 78, 102, 196, 433, 461 from Colbourne et al. 2004, Cristescu et al. 2006) were amplified by adding 1µl of extracted DNA to 6µl of Qiagen multiplex PCR mastermix, 1.2µl of Dp primer mix, and 3.8µl of sterile molecular grade water. Mixtures were amplified on a DNA engine Dyad Thermal Cycler starting with 1 cycle at 95°C for 15 minutes followed by 30 cycles of 94°C for 30 seconds, 50.5°C for 180 seconds, and 72°C for 90 seconds with a final extension at 72°C for 10 minutes. Amplified DNA was diluted to 1µl of DNA and 10µl of sterile molecular grade water for fragment analysis at the Keck Center for Comparative and Functional Genomics at the University of Illinois at Urbana-Champaign Biotechnology Center (Urbana, IL, USA). Alleles were called visually through GeneMapper software (Version 5: Applied Viosystems, Foster City, CA, USA).

Appendix B: Sample size for field genotyping and Juvenile Growth Rate (JGR) assays.

Table B.1. Sample sizes for the genotyping and juvenile growth rate (JGR) assays. Each is grouped by population and then season of collection. Numbers genotyped and assayed are given for each individual year. We increased sample size after the first season of collection (August 2011).

Population	Season	Year	Number Genotyped / Number Unique	Clonal Richness	Frequency of dominant genotype	Unique genotypes assayed
Non-persisting populations						
Baker	Spring	2012	18 / 11	0.61	17%	7
		2013	46 / 21	0.46	15%	6
Cloverdale	Spring	2012	31 / 12	0.38	23%	8
		2013	36 / 25	0.69	14%	9
Little Long	Spring	2012	38 / 29	0.76	8%	4
		2013	42 / 40	0.95	5%	15
Persisting populations						
Bassett	Spring	2012	111 / 31	0.30	38%	6
		2013	49 / 29	0.59	10%	10
	Summer	2011	15 / 13	0.87	15%	5
		2012	53 / 28	0.53	21%	11
Bristol	Spring	2012	64 / 47	0.73	8%	8
		2013	30 / 20	0.67	17%	3
	Summer	2011	13 / 12	0.92	15%	5
		2012	77 / 44	0.57	5%	8
Warner	Spring	2012	82 / 42	0.51	12%	13
		2013	48 / 24	0.50	17%	11
	Summer	2011	16 / 6	0.38	38%	6
		2012	55 / 24	0.44	20%	5

Appendix C: Supplementary figures for Chapter 1.

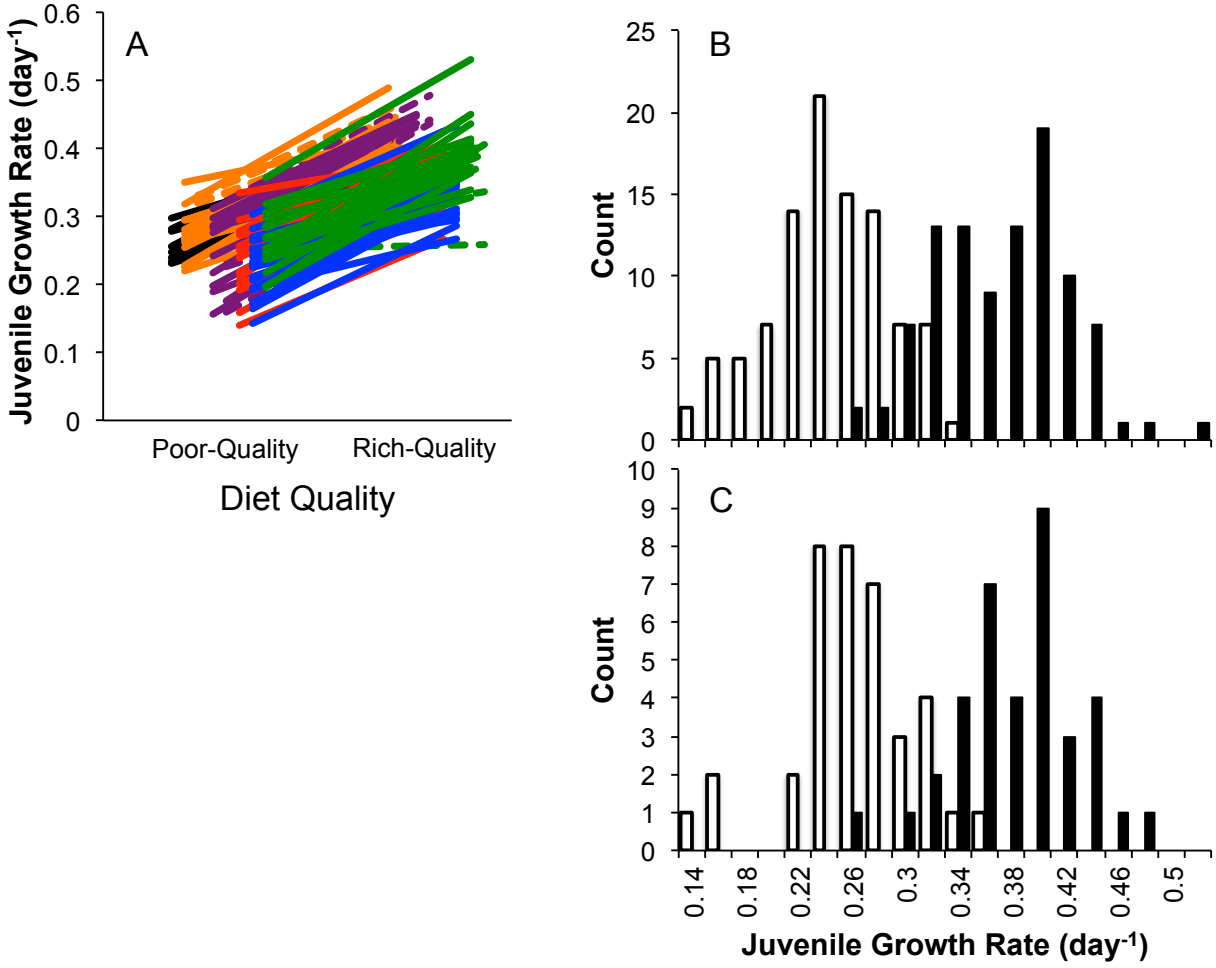

Figure C.1. (A) Reaction norms for juvenile growth rate (JGR) on poor- and rich-quality diets. Each line connects a genotype's average JGR on *Oocystis* (left, poor-quality) and *Ankistrodesmus* (right, rich-quality). The x-axis is also offset so that individual populations are visible. Colors represent individual lakes (from left to right: Black = Baker, Orange = Bassett, Purple = Bristol, Red = Cloverdale, Blue = Little Long, Green = Warner). Solid lines are genotypes that were collected in the spring and dashed lines – offset to the right – are the genotypes that were collected in the summer. (B & C) Histograms illustrating the range of variation in growth rates on both diets (rich-quality = solid bars; poor-quality, open bars) for the clones depicted in Panel A, (B) is the spring-collected clones and (C) is the summer-collected clones.

Appendix D: Analysis of spring and summer investment in sexually-produced dormant offspring.

Table D.1. Analysis of variance for investment in sexual reproduction by spring- and summer-collected individuals from the three persisting populations. Models were run using SAS Version 9.4, Proc GLM with population of collection and season of collection (nested in population).

Source	d.f.	Mean Square	*F*-Value	*P*-Value	
Sum investment in sexually-production and dormant offspring					$R^2 = 0.047$
Population	2	0.012	4.11	0.138	
Season (Population)	3	0.003	0.44	0.722	
Residual	112	0.077			
Investment in male offspring					$R^2 = 0.026$
Population	2	0.004	5.64	0.096	
Season (Population)	3	0.001	0.25	0.863	
Residual	112	0.003			
Investment in Ephippia					$R^2 = 0.122$
Population	2	0.016	8.49	0.058	
Season (Population)	3	0.002	0.70	0.555	

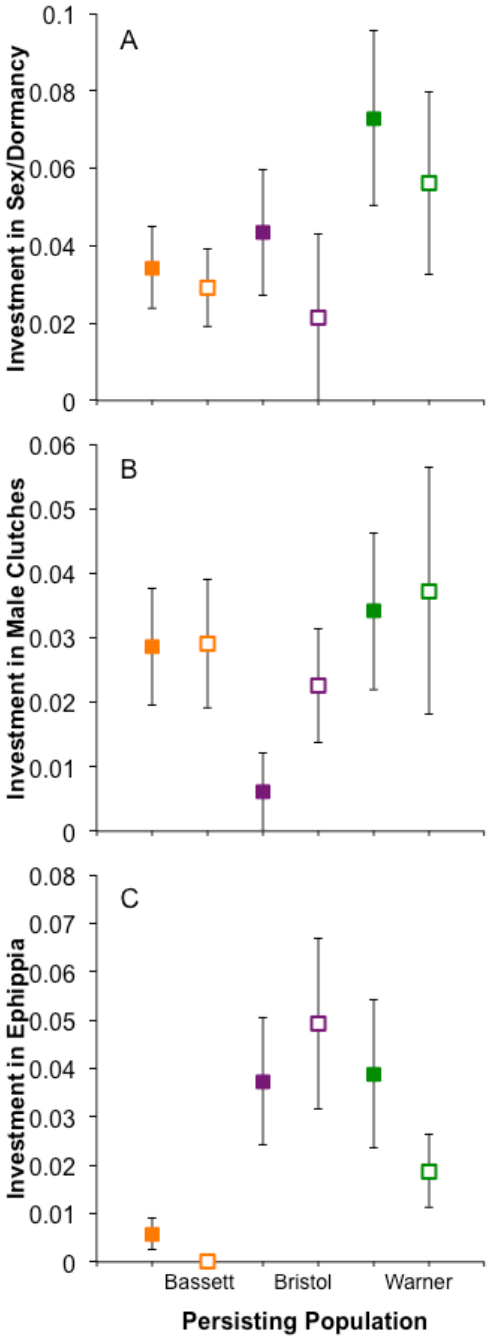

Figure D.1. No difference in the three metrics of sex/dormancy investment between populations or seasons in the three persisting populations. (A) Sum investment in male and ephippial clutches, (B) male investment, or (C) ephippia investment. Populations are grouped with mean May-collected investment on the left (closed squares) and mean August-collected investment on the right (open squares). Note the low proportion of investment compared to Cloverdale or Little Long (Fig. 2.1).

Appendix E: Field profile of chlorophyll-*a* abundance in Sportsman's Lake.

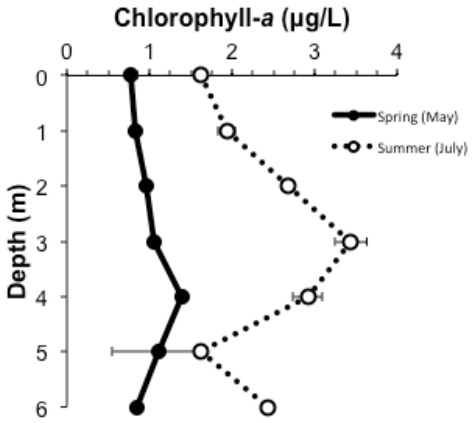

Figure E.1. Profile of chlorophyll-*a* abundance in the spring (solid line, closed circles) and summer (dashed line, open circles). Each point is the mean of 2 replicate samples with 1 standard error. Maximum chlorophyll-*a* was at 4m in May and 3m in July. On sampling for the laboratory assay of growth on field-collected resources, we sampled 1m below the chlorophyll-*a* maxima. We calculated the area under the curve (using the trapezoid rule) past the chlorophyll-*a* maxima. We found that the sum area of chlorophyll-*a* available between 0m and 5m in May was 5.185µg/L-m (which converts to 1.037µg/L averaged across the 5m sample). The sum area for chlorophyll-*a* available between 0m and 4m in July was 10.325µg/L (which converts to 2.581µg/L averaged over the 4m sample). Both of these values were similar to the chlorophyll-*a* abundances (collected via integrated tube sample) that we fed to the *Daphnia* in the JGR assay.

Appendix F: Additional methods of genotyping for the competition assay.

We assessed unique genotypes through analysis of microsatellites (see Cristescu et al. 2006, Allen et al. 2010, Holmes et al. 2016; Chapter 1). We used Qiagen DNeasy Blood & Tissue Kit for whole animal tissue digestion and DNA extraction. Individuals were digested in a 20µl[proteinase K]:180µl[ATL Tissue Lysis Buffer]. Individuals were then incubated at 37°C for a minimum of four hours and a maximum of 17 hours. DNA was extracted using a 400µl equal mixture of EtOH and AL lysis buffer and centrifuged in a Qiagen spin column. DNA was washed twice with Qiagen AW1 and AW2 with centrifugation after each wash. AE elution buffer (30µl) was used to remove the DNA into a clean tube.

We used six microsatellite loci for the *Daphnia pulex* (Dp) complex: DP 27, 78, 102, 196, 433, 461 (Colbourne et al. 2004, Cristescu et al. 2006). We amplified our DNA on a SimpliAmp ThermoCycler (Applied Biosystems by Life Technologies) by adding 1µl of DNA, 6µl Qiagen multiplex PCR mastermix, 1.2µl Dp primer mix, and 3.8µl sterile molecular grade water. Cycles began with 1 cycle at 95°C for 15 minutes followed by 30 cycles of 94°C for 30 seconds, 50.5°C for 180 seconds, and 72°C for 90 seconds. We included a final extension at 72°C for 10 minutes. Amplified DNA was diluted with 10µl of sterile molecular grade water to 1µl DNA/primer-mixture and sent for fragment analysis at the Keck Center for Comparative and Functional Genomics at the University of Illinois at Urbana-Champaign Biotechnology Center (Urbana, IL, USA). We visually called alleles using GeneMapper software (Version 5, Applied Vivosystems, Foster City, CA, USA) specifically looking for alleles that differed between the two competing genotypes.

Appendix G: Monoculture and competition population densities in the 21-day laboratory competition assay.

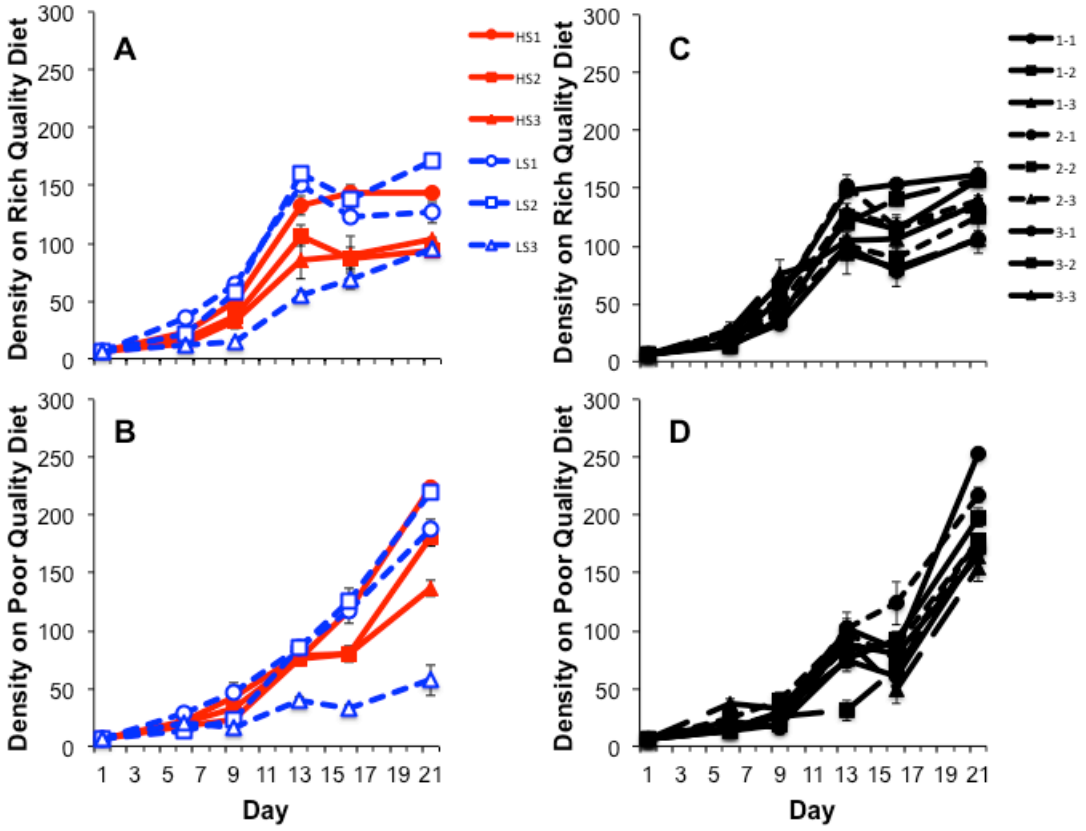

Figure G.1. Monoculture (A and B) and competition (C and D) densities across the 21-day experiment in each of the diet treatments. Each point is the mean of three replicates with 1 standard error. Note the plateau in density in the rich-quality treatment (A and C) that occurs at day 13. In monoculture, colors denote sensitivity to resource quality (high sensitivity: red; low sensitivity: blue). In competition, total density is expressed by the line. The key denotes the competition combination listing first the high sensitivity genotype followed by the low sensitivity genotype.